DIAGNOSTIC UTILITY OF SQUASH CYTOLOGY AND ITS CORRELATION WITH HISTOPATHOLOGY IN NEUROPATHOLOGICAL SPECIMENS

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DECLARATION

I hereby declare that the dissertation entitled **"DIAGNOSTIC UTILITY OF SQUASH CYTOLOGY AND ITS CORRELATION WITH HISTOPATHOLOGY IN NEUROPATHOLOGICAL SPECIMENS"** was done by me in the Department of Pathology at Coimbatore Medical College & Hospital, Coimbatore during the period from March 2010-June 2011, under the guidance and supervision of **Dr. C. LALITHA**, **M.D.,** Additional Professor, Department of Pathology, Coimbatore Medical College, Coimbatore. This dissertation is submitted to the Tamilnadu Dr. M.G.R. Medical University, Chennai towards the partial fulfillment of the requirement for the award of M.D., Degree in Pathology. I have not submitted this dissertation on any previous occasion to any University for the award of any degree.

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CERTIFICATE

This is to certify that the dissertation entitled **"DIAGNOSTIC UTILITY OF SQUASH CYTOLOGY AND ITS CORRELATION WITH HISTOPATHOLOGY IN NEUROPATHOLOGICAL SPECIMENS"** is a record of bonafide work done by **Dr. S. Jamuna Rani,** Post graduate student in the Department of Pathology, Coimbatore Medical College and Hospital, Coimbatore, under the supervision of **Dr. M. MURTHY, M.D.,** Professor & Head, Department of Pathology, Coimbatore Medical College and Hospital, and under the guidance of **Dr. C. LALITHA, M.D.,** Additional professor, Coimbatore Medical College and Hospital, in partial fulfillment of the regulations of the Tamilnadu Dr. M.G.R. Medical University towards the award of M.D. Degree (Branch III) in Pathology.

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CONTENTS

LIST OF TABLES

LIST OF CHARTS

LIST OF COLOR PLATES

21.	Reticulin staining in Hemangioblastoma
22.	CD34 immuno staining in Hemangioblastoma
23.	Squash cytology in Pleomorphic xanthoastrocytoma
24.	Histopathology in Pleomorphic xanthoastrocytoma
25.	Squash cytology in Ependymoma
26.	Histopathology in Myxopapillary ependymoma
27.	Squash cytology in Non neoplastic lesion
28.	Histopathology in Non neoplastic lesion
29.	Gomori's methanamine silver staining in Aspergillus

ABSTRACT

DIAGNOSTIC UTILITY OF SQUASH CYTOLOGY AND ITS CORRELATION WITH HISTOPATHOLOGY IN NEUROPATHOLOGICAL SPECIMENS

INTRODUCTION

Brain is the most vital organ in our body. Unlike other systems of the body, lesions in central nervous system (CNS) cannot be localised with specific symptoms or signs. It needs an integrated approach. It involves the neurophysician, neurosurgeon, radiologist and pathologist. It is the pathologist who gives the final report and the treatment is based on the pathologist's report. Hence, it is of paramount importance to diagnose CNS lesions with accuracy.

Background of the study

Squash preparation is an effective, simple, rapid, relatively safe, and reliable technique for the diagnosis of central nervous system tumors. The knowledge of the squash preparation technique could be beneficial in centers where a facility for frozen sections is unavailable, in case of a power break-down, or a lack of trained technical personnel. Squash cytology is very useful in CNS lesions due to the intrinsic soft consistency of the brain tissue.

In the era of stereotactic biopsies, where the amount of tissue fragment is very small, good preservation of fine cellular details can be obtained with squash cytology. Squash cytology is not affected by edema, hemorrhage, necrosis or calcification. The current study was undertaken to assess the diagnostic utility of squash cytology and to correlate with histopathological diagnosis.

AIM OF THE STUDY

The aim of the study is to assess the diagnostic utility of squash cytology and to correlate with histopathology in neuropathological specimens.

MATERIALS AND METHODS

This is a prospective study. The study was conducted in the Department of Pathology, Coimbatore Medical College, Coimbatore. The study was conducted after obtaining the ethical approval from the Ethical Review Committee of Coimbatore Medical College, Coimbatore. Fifty cases of Neuropathological specimens received between from March 2010 to June 2011 were assessed. The study subjects were patients admitted to the Department of Neurosurgery for space occupying lesions of brain and spinal cord. During surgery, upon opening the lesion, small bits of tissue measuring 1-2mm^2 were taken in a fresh state and sent for squash cytology in gauze moistened with saline. The remaining tissues were fixed in 10% formalin and sent later for histopathology. In difficult cases where conclusion could not be made even with histopathology, special stains and immunohistochemistry were used as adjuvants to histopathology. Whenever there was difficulty in grading astrocytomas, tumor grading was done with Ki67/MIB-1 labelling index using immunohistochemistry.

SUMMARY

The prevalence of CNS lesions reported at Department of Pathology, Coimbatore Medical College was 1.1%. The sensitivity of squash cytology in detecting CNS tumors was 97.62%. The specificity of squash cytology in detecting CNS tumors was 75%. The positive predictive value of squash cytology was 95.35%. The negative predictive value of squash cytology was 85.71%. The percentage of false positive results was 25%. The percentage of false negative results was 2.38%.

CONCLUSION

Squash cytology is a sensitive and specific modality for diagnosing space occupying lesions of brain and spinal cord. The method is easy, rapid and inexpensive. Details of cellular morphology are well seen in squash cytology. Hence squash cytology can be used as a reliable diagnostic tool in developing countries like India, since the cost of cryostat is prohibitive and unlike cryostat, squash cytology does not require any electricity for slide preparation. Moreover, cryostat needs an experienced microtomist to cut the sections and the problems of freezing artefacts cause diagnostic pitfalls.

Despite the advantages, Squash cytology should be used as a preliminary investigation and should always be confirmed with Histopathology which is the golden standard. It should never be used solely for diagnostic or therapeutic purposes.

KEY WORDS

- Diagnostic Utility
- Squash Cytology,
- CNS Tumors
- Histopathology
- Brain Tumor.

INTRODUCTION

Brain is the most vital organ in our body. It integrates all the systems of our body. Brain is important for the co-ordinated function of the body. Space occupying lesions of the brain include congenital anomalies, hamartomas, vascular malformations, inflammatory demyelinating disorders, infections and tumors. Unlike other systems of the body, lesions in central nervous system (CNS) cannot be localised with specific symptoms or signs. It needs an integrated approach. It involves the neurophysician, neurosurgeon, radiologist and pathologist. It is the pathologist who gives the final report and the treatment is based on the pathologist's report. Hence, it is of paramount importance to diagnose CNS lesions with accuracy.

EPIDEMIOLOGY OF CNS TUMORS

The overall incidence of CNS tumors including children and adults is 2%[1]. In children, tumors of the nervous system are the second most common tumors next to leukemias and the most common solid tumors[2]. The CNS malignancies represent 16.6%[2] of all malignancies during childhood and adolescence in the world. In India, tumors of the CNS constitute 11.3%[2] in children[3].

According to the SEER reports[4], the incidence of CNS tumors is increasing with more than 4000 new cases diagnosed annually. Worldwide, the incidence rate of primary malignant tumors of the central nervous system ranges from 5.8 per 100 000 person-years in males and 4.1 per 100 000 person-years in females in developed countries to 3.0 per 100 000 person-years in males and 2.1 per 100 000 in females in developing countries. In adults, CNS tumors constitute the sixth most common form of tumor. In India, the

incidence in adult males is 11.2/100000 and in adult females is 6.8/100000. The age adjusted mortality is 4.5/100000[5].

Though CNS tumors constitute only less than 2%, the symptoms associated with tumors are very distressing. The symptoms range from headache to crippling paraparesis culminating in death of the patient. The time gap between the point of detection of a CNS tumor and the point at which it becomes incurable is very narrow.

Advances in neuroimaging techniques permit early and exact localisation of the tumors. The stereotactic brain biopsies permit accurate sampling of multiple areas. Hence, it is essential to examine minute tissue samples and give accurate pathology reports at the earliest.

Similarly, non neoplastic lesions, especially infective lesions like tuberculomas, brain abscess, fungal infections, neurocysticercosis should be diagnosed early as they are potentially curable. But, if left untreated or if there is a delay in diagnosis, it can cause permanent neurological deficits.

There are two methods available for cytological diagnosis of CNS lesions. One is squash cytology and other is cryostat sections. Squash cytology provides rapid and efficient means for diagnosing nervous system lesions. In experienced hands, it is capable of obtaining high degree of diagnostic accuracy. Moreover, squash cytology does not need any sophisticated equipments like cryostat. It does not need a microtomist to cut sections. It is simple and reproducible.

Despite the advances, there are a few limitations in squash cytology. It cannot be performed for calcified tissue. If too much pressure is exerted between two slides, it causes crush artefacts which pose problems in interpreting the smear.

Despite the limitations, Squash cytology is relatively accurate, safe, rapid and simple. However, squash cytology should always be correlated with Histopathology, which is the golden standard for diagnosis.

NEED FOR THE STUDY

Squash preparation is an effective, simple, rapid, relatively safe, and reliable technique for the diagnosis of central nervous system tumors. The knowledge of the squash preparation technique could be beneficial in centers where a facility for frozen sections is unavailable, in case of a power break-down, or a lack of trained technical personnel. Squash cytology is very useful in CNS lesions due to the intrinsic soft consistency of the brain tissue.

In the era of stereotactic biopsies, where the amount of tissue fragment is very small, good preservation of fine cellular details can be obtained with squash cytology. Squash cytology is not affected by edema, hemorrhage, necrosis or calcification. The current study was undertaken to assess the diagnostic utility of squash cytology and to correlate with histopathological diagnosis.

AIM OF THE STUDY

The aim of the study is to assess the diagnostic utility of squash cytology and to correlate with histopathology in neuropathological specimens.

OBJECTIVES OF THE STUDY

- To assess the diagnostic utility of squash cytology in neuropathological specimens.
- To assess the diagnostic accuracy of squash cytology by correlating with histopathology.
- To determine the sensitivity and specificity of squash cytology in CNS tumors.
- To assess the utility of squash cytology in predicting the grade of CNS tumors.
- To determine the diagnostic limitations of squash cytology.
- To study the age and sex incidence of CNS tumors and comparing with global statistics.

REVIEW OF LITERATURE

Tumors of the nervous system are the second most common form of cancer in children and the sixth most common form of cancer in adults[1]. Approximately 4400 people are newly diagnosed with a brain tumour each year across the world. However according to the Indian council of medical research project CNS tumors are the second most common tumors in children between 0-14 years of age[2]. The incidence is 16.6% in the world and it is around 11.3%[3] in India.

Though tumors of brain and spinal cord constitute less than 2% of all cancers[3], the symptoms and signs associated with these tumors can be very distressing to the patients. They also cause increased mortality and decreased life expectancy. Infective lesions like brain abscess, tuberculosis and fungal granulomas are also noteworthy. Early and correct diagnosis of these lesions is essential as they are completely curable. At the same time, delay in treatment or mismanagement can increase the mortality. With increasing use of CT and MRI, tumors and other lesions in CNS are diagnosed early and hence definitive diagnosis must be given for further treatment and management.

The role of squash cytology in neuropathological practice is very crucial. The usefulness of squash cytology has increased with the development of stereotactic brain biopsies where the amount of tissue available is very limited. However, definitive diagnosis must be given for effective management.

Squash cytology is a simple, accurate and reliable procedure. In experienced hands, the diagnostic accuracy is around 92-94%[6].

SQUASH CYTOLOGY

Rapid cytological diagnosis in general pathology is dependent mainly on cryostat sections and touch imprints. But in CNS, squash cytology smear is a very useful technique due to the general soft and intrinsic consistency of CNS lesions which facilitates the preparation of smear.

History of Squash Cytology

Squash cytology preparation was first introduced by Eisenhardt and Cushing[7] in early 1930. They used supravital staining to visualise the tissues. In supra vital staining the tissue is stained in a live state. Later, the technique was modified by Badt in 1937 where toluidine blue was used to stain the tissue. This technique was further championed and documented by Russell et al., in 1937[8]. The present technique was introduced by Russell along with Sir Hugh Cairns.

Requisition for rapid cytological diagnosis

- The main aim of stereotactic biopsy is to obtain enough tissue so that further diagnosis with paraffin sections can be done.
- For definitive Neurosurgical management.
- When an unexpected lesion is encountered during the procedure, rapid cytological diagnosis helps the surgeon to modify the approach.
- Status of resected margins for radical excision.

The importance of Clinical History

It is of paramount importance for the pathologist to know the age, sex of the patient, location of the lesion, whether the lesion is intra-axial or extra-axial and the

radiological findings. Other useful information include the type and duration of clinical symptoms. For example, a history of seizure[9] is suggestive of a slow growing lesions such as low grade tumor whereas signs of elevated intracranial pressure is associated with high grade tumor. History of previous surgery, chemotherapy and radiotherapy are also important.

INVESTIGATIVE MODALITIES IN CNS LESIONS

Imaging Techniques

They are the first line of investigations in any patient with CNS symptoms. They include CT, MRI and PET scan (Positron Emission Tomography). MRI is more sensitive than CT in evaluating posterior fossa lesions and in detecting infiltration of the cerebral parenchyma. However, CT is superior to MRI in detecting calcified lesions. Cerebral and spinal angiography are used for vascular lesions.

Methods for rapid cytological diagnosis

For rapid cytological diagnosis of CNS lesions, the following methods are available,

- Squash Cytology.
- Imprint Cytology.
- Cryostat Sections.

Out of these methods squash cytology is the most cost effective, fastest, most reliable and accurate for CNS lesions[10]. In stereotactic biopsy, where only 1-2mm^2 of tissues are available, squash cytology is the best method.

Comparison of squash cytology and Cryostat sections

Squash Smears	Cryostat Sections
No special equipment is needed	Needs a cryostat
Quick and easy to prepare	Needs expertise in preparing sections
Cytological and nuclear details well preserved	Architectural details well preserved
Crush artefacts are common problem	Freezing artefacts are common problem

If the material obtained is largely calcified (as in spinal cord meningioma) then neither squash smear nor cryostat is possible. In such cases, imprint cytology can be made which will allow a diagnosis based on tumor cell cytology.

Biopsy

Though there are many modalities for diagnosing CNS lesions, none of the techniques allows a specific diagnosis to be made with absolute certainty except histopathology. Hence, permanent Haematoxylin and Eosin sections are the golden standard in establishing the diagnosis[11].

Ancillary Investigations

Ancillary investigations include special stains, immunohistochemistry and molecular genetic analysis.

Special stains include reticulin stain for hemangioblastoma, hemangiopericytoma and lymphoma, Periodic acid Schiff and Mucicarmine for metastatic carcinoma and Van

Gieson for dural infiltration in meningioma. Vonkossa stain for calcium can demonstrate psammoma bodies in meningioma and calcification in oligodendroglioma. In infective lesions, fungal stains such as Gomori's methanamine silver demonstrate fungal organisms. In Cryptococcus, the capsule can be demonstrated by mucicarmine staining which stains the capsule of the organism.

Immunohistochemistry

Immunohistochemistry is used to investigate cellular differentiation in CNS tumors and also as an independent prognostic tool. Most commonly used markers are Glial Fibrillary Acidic Protein, S-100, Epithelial membrane antigen and Neuro filament protein. In practice, Ki-67/MIB-1 is the most frequently used marker in CNS tumors. It is used to detect proliferating cells and is of use in grading gliomas which is of prognostic significance.

Molecular Genetic Analysis

Analysis of genetic alterations can provide important clinical and prognostic information. Oncogene activation can be detected by southern blotting, polymerase chain reaction and fluorescence in situ hybridisation. Mutations of TP53 gene on chromosome 17p13.1 and loss of heterozygosity of chromosome 17p are seen in more than half of Gliomas[12]. Genetic analysis is an important diagnostic tool in childhood neuroblastomas as NMYC amplification is closely related with rapid clinical course and worst prognosis. With advent of molecular genetics, the next decade will see a targeted approach to brain tumors in the form of immuno therapy, cancer stem cell therapy and epigenetic therapy[13].

Distribution of Smear Tissue

After smearing the tissue, the smear is examined macroscopically for the distribution of tissue before fixation as this can give an indication whether the tumor tissue is present or not. Normal brain tends to spread out in a uniform monolayer where as intrinsic neoplasms, metastasis and meningiomas spread in an irregular aggregated fashion. Some tumors like schwannomas and hemangioblastomas are difficult to smear and are best investigated by cryostat sections.

INTERPRETATION OF SMEAR PREPARATION

The use of smear technique for rapid pathological diagnosis of CNS lesions provide an efficient means of pathological assessment which in experienced hands can give a high degree of accuracy. Before attempting to interpret the smear, it is essential to know the normal cytology of CNS in smear preparation.

NORMAL CYTOLOGICAL APPEARANCE

Cerebral Cortex

Cerebral cortex biopsies contain large neurons with pyramidal cells, small compact neurons and glial cells. Astrocytes have large ovoid nucleus with speckled chromatin and variable cytoplasm. Normal cerebral cortex should not be misinterpreted as ganglion cell tumor.

White Matter

White matter comprises of nuclei of oligodendrocytes, astrocytes with occasional microglia. The background stains intensely due to the abundance of myelinated fibres.

Cerebellar Cortex

Smear preparation of cerebellar cortex shows a characteristic population of small round uniform intensly staining nuclei of granular cells and large Purkinje cells with branching dendritic tree. This should not be mistaken for metastatic small cell carcinoma, lymphoma or medulloblastoma.

Choroid Plexus

The epithelium is uniform in size and shape. The cells are densely cohesive around the core of thick fibrovascular core. This is often mistaken for choroid plexus papilloma.

Arachnoid Cells

They appear as rounded or whorled collections of polygonal cells with ill defined cytoplasm. These may be mistaken for meningioma.

REACTIVE CHANGES IN SMEAR PREPARATIONS

Reactive Gliosis

May be misinterpreted as glioma if attention is focussed on the cytoplasmic features and not on the cellular density and distribution. Well differentiated astrocytomas are hypercellular and the cells are irregularly distributed. Unlike glioma cells, the cells of reactive astrocytosis have stellate configuration with long tapering radiating processes. They are hypertrophic, but not increased in number.

Reactive Microglia

They are seen as rod cells in smear with elongated nuclei and scanty cytoplasm.

Acute Inflammatory Conditions

Acute inflammatory cells, mainly neutrophilic polymorphs are seen in cerebral abscess, cerebritis, early stage of cerebral infarction and tumor necrosis.

Chronic Inflammatory Conditions

They are characterised by perivascular lymphocytic cuffing which is very well demonstrated in squash preparations. Multinucleated giant cells and epithelioid cells are seen in tuberculous infection. In cryptococcal infection, there are numerous macrophages which can occasionally contain the organism.

Artefact

Artefacts can arise at several stages in preparation and staining of smears. They can hinder the diagnosis by distorting the cytological features.

ARTEFACT	MISTAKEN INTERPRETATION
Crush artefact	Fibrillary astrocytoma
Thick smear	Malignant Glioma
Bone dust	Calcospherites
Starch powder	Psammoma Bodies
Hemostatic sponge	Calcification
Drying artefact	Necrosis/Mitosis/Apoptotic Figure

Approach to CNS tumors in Squash preparation

When evaluating a lesion by cytological smear, attention must be paid to the following details,

- Relationship of tumor cells to blood vessels.
- Type of blood vessel.
- Type of background.
- Study of relationship of cells to each other.
- Nuclear and cytological details.
- Evidence of necrosis/ mitotic activity.

Relationship of Tumor cells to Blood vessels

Astrocytomas demonstrate aggregation of tumor cells close to blood vessels in a perivascular gradient pattern.

Lymphoma can infiltrate blood vessel walls, but often dispersed in a discohesive fashion away from blood vessels in a angiocentric and diffuse pattern.

Metastatic carcinoma cells are distributed close to and away from blood vessels with or without vascular affinity.

Type of Blood Vessel

Thin walled blood vessel is seen in Oligodendrogliomas, grade 2 and grade 3 astrocytomas, metastatic carcinomas and lymphomas. Vessels with endothelial proliferation are characteristically seen in glioblastoma multiformae.

Type of Background

Normal brain matter is characterised by a felt like background. Astrocytomas usually demonstrate the presence of fine, well defined glial processes in the background known as fibrillary background. Reactive gliosis also demonstrate the fibrillay background. But in Metastatic carcinoma and lymphoma, the fibrillary background is characteristically absent. Instead, felt like pattern is seen.

Study of Relationship of cells to each other

Glial tumors and lymphomas are dispersed as monolayered sheets of cells where as metastatic carcinomas contain irregular clusters of cells.

Nuclear and Cytological details

Nuclear and cytological details are seen at high magnification. Nuclear pleomorphism and atypia are noted. In addition, the presence of specialised features such as astrocytic processes, gliofibrillary matrix, cellular whorls, psammoma bodies and papillary structures are also noted.

Evidence of Necrosis and Mitosis

Necrosis is common in glioblastomas and metastatic carcinomas. But this should not be confused with cerebral infarction or active demyelination. Mitotic figures can occur in CNS neoplasms, reactive gliosis and in proliferating capillary endothelium at the edges of infarct.

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TUMORS OF THE CNS

Tumors of CNS can be classified according to the cell of origin into five groups

1. Neuroectoderm, principally gliomas.
2. Mesenchymal structures, notably meningiomas and schwannomas.
3. Ectopic tissues, such as craniopharyngiomas, dermoid and epidermoid cysts, lipomas and dysgerminomas.
4. Retained embryonal structures such as paraphyseal cysts.
5. Metastasis.

Histologic versus Biologic Malignancy

The terms benign and malignant require qualification when used in reference to brain tumors.

- Histologic malignancy is determined by the tumor's ability to cause ill effects based upon its cellular anaplasia (i.e., mitotic figures, hyperchromasia, pleomorphism) and its ability to invade and metastasize.
- Biologic malignancy is based upon the tumor's ability to cause ill effects based upon its location. For example, a histologically bland noninvasive meningioma can still be lethal, based upon its ability to expand within a confined space and eventually cause herniation.

Age and Sex

The age distribution of primary CNS neoplasm is bimodal, with first peak in children (eg. Medulloblastoma and pilocytic astrocytoma) and a second large peak in adults aged 45-70 years, mainly due to gliomas.

The incidence of invasive CNS tumors is higher in males than females and higher among white children than black children. There is a general tendency for gliomas and embryonal tumors to occur more frequently in males, whereas meningiomas preferentially affect females. In women, more than 50 percent of primary intracranial tumors are meningiomas. In spinal meningiomas, the preferential occurrence in women is even stronger, with male to female ratio as low as 0.15%[14].

Location of Brain Tumors

Most of the intracranial tumors have predictable geographic locations. For example, astrocytic neoplasms and oligodendrogliomas occur predominantly in the middle cranial fossa where as ependymomas have their highest incidence in the fourth ventricle. Paradoxically, Ependymomas are the least common in the lateral ventricles, which have the largest ependymal surface. Meningiomas arise from widely distributed arachnoidal cells along the meninges. Majority of the CNS tumors in children arise in the posterior fossa in relation to the brain stem and cerebellum.

ETIOLOGIC FACTORS IN CNS NEOPLASM

Environmental Factors

Occupational and environmental exposures have been implicated in CNS tumors. Farmers and petrochemical workers have been shown to have a higher incidence of primary brain tumors due to exposure to pesticides. Occupational exposure to formaldehyde can cause CNS tumors.

Ionizing and Nonionizing Radiation

Meningiomas arise in individuals receiving cranial or scalp irradiation. There is a 2.3% incidence of primary brain tumors in long-term survivors among children given prophylactic cranial irradiation for acute leukemia.

Nonionizing radiation emitted by cellular phones has become a topic of considerable controversy these days. Cellular phones use radio frequency waves that fall between radiowaves and microwaves. According to Frumkin et al study[15] radio frequency wave exposure is related to duration and frequency of cellular phone use. But Inskip et al

study[16] has come to the opposite conclusion that there is no correlation between cellular phone usage and brain tumor development.

Viral Associations

There is no clear association between primary brain tumors and viruses. Simian virus 40 contaminating older polio vaccine preparations cause brain tumors[17]. Primary CNS lymphoma has been shown to be associated with Epstein-Barr virus[18]. The majority of primary CNS lymphomas are B-cell large immunoblastic, and Epstein-Barr DNA is identifiable in nearly all cases. An increase in incidence of primary CNS lymphoma is most likely due to the increasing numbers of immunosuppressed patients with human immunodeficiency virus.

Hereditary Syndromes

Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder affecting 1 in 3,000 individuals. It is commonly associated with schwannomas. Other CNS tumors such as optic gliomas, astrocytomas, and meningiomas also occur at significantly higher frequency in patients with NF1. NF2 is far less common, occurring in approximately 1 in 35,000 individuals and is characterized by bilateral vestibular schwannomas and meningiomas.

Other hereditary tumor syndromes affecting the CNS include Li-Fraumeni syndrome (germline mutation in p53 allele), Von Hippel-Lindau syndrome (germline mutation of the VHL gene) and Turcot's syndrome (germ line mutations of the adenomatous polyposis gene). The nevoid basal cell carcinoma syndrome (Gorlin's syndrome) is associated with medulloblastomas and meningiomas and represents mutations in the PTCH suppressor gene or other members of the sonic hedgehog signaling pathway.

GRADING OF NERVOUS SYSTEM TUMORS

Tumors affecting the brain and spinal cord are diverse and many grading systems were used. The first attempt for grading the CNS tumors was done by Percival Bailey and Harvey Cushing based on embryonic derivation of tumor cells[19]. Subsequently, in 1949, Kernohan and Sayre proposed a four tier system based on tumor's degree of dedifferentiation and prognosis of the patients. Ringertz proposed a three tier scheme at the same time and classified gliomas as astrocytoma, anaplastic astrocytoma and Glioblastoma multiformae. Russell and Rubinstein continued to modify and update the Bailey and Cushing system from the 1960s to 1980s[20]. The current World Health Organization (WHO) classification scheme was first completed in 1979 and then revised in 1993, 2000, and 2007. This scheme is currently the most widely utilized for tumor typing and grading[21]. The WHO classification is given in Annexure II.

ASTROCYTIC TUMORS

Astrocytomas are the most common primary brain tumors in adults. They constitute 30% of all CNS tumors in adult population[22]. They are composed of cells that morphologically resemble astrocytes and express Glial fibrillary acidic protein (GFAP). Many grading systems are used for astrocytomas. However, the WHO grading system and the St.Anne Mayo grading are the most widely used.

Comparison of grading scheme for Astrocytoma

Kernohan 1949	Bailey and Cushing[19]	Ringertz Current	WHO Current[26]	St-Anne Mayo
1	Astrocytoma	Astrocytoma	II	2
2	Astroblastoma	Anaplastic Astrocytoma	III	3
3	Spongioblastoma Multiformae	glioblastoma	IV	4

In St-Anne Mayo scheme, the specimen is assessed for the presence of four variables,

1. Nuclear atypia.
2. Mitosis.
3. Endothelial proliferation.
4. Foci of coagulative necrosis.

Accordingly, tumors are graded as grade1 if none of the variables are present, grade 2 if one variable is present, grade 3 if two variables are present and grade 4 if three or all four variables are present. Though St-Anne Mayo scheme is simple and reproducible, this rigid classification places a low grade tumor in a high grade category.

Now-a-days the WHO grading system is most widely used

Grade 1 : Benign tumor (Pilocytic astrocytoma).

Grade 2 : High degree of cellular differentiation, slow growth and diffuse infiltration into adjacent brain parenchyma.

Grade 3 : Anaplasia (increased cellularity, pleomorphism and mitosis).

Grade 4 : Endovascular proliferation and necrosis.

Grade II Astrocytoma

Grade II astrocytomas include fibrillary, protoplasmic and gemistocytic variants. Among these, fibrillary astrocytomas are the most common variant[23]. Grade II astrocytomas typically occur in the fourth decade of life with male to female ratio of 1.18-1[24]. Majority occur in cerebral hemispheres. Grossly they cause diffuse gyral expansion with effacement of the normal structures. They are predominantly solid with focal cystic areas.

Fibrillary Astrocytoma

In smear preparations, fibrillary astrocytomas typically demonstrate fibrillary background. The neoplastic astrocytes are enlarged with hyperchromatic nuclei and coarse chromatin are seen.

In paraffin sections, fibrillary astrocytomas typically show dyscohesive cells composed of neoplastic astrocytes with hyperchromatic pleomorphic nuclei in a patternless array of fibrillary background. However, the distinction between reactive gliosis and low grade astrocytoma is difficult.

DISTINCTION BETWEEN LOW GRADE ASTROCYTOMA AND REACTIVE GLIOSIS

Low grade Astrocytoma	Reactive Gliosis
1.Uneven distribution of neoplastic Astrocytes.	1.Even distribution of hyperplastic Astrocytes.
2.Microcystic change and calcospherites favour neoplastic.	2. Foamy macrophages favour benign process.
3.Lymphocye cuffing is rare in malignancy except in gemistocytic astrocytoma and pleomorphic xanthoastrocytoma.	3.Lymphocyte cuffing of regional blood vessels in inflammatory lesions.
4. GFAP expression for astrocytoma reveals rich fibrillary matrix[23].	4. GFAP expression typically reveals cytoplasmic extensions in a radial, stellate configuration around the hyperplastic astrocytes[25].

Gemistocytic Astrocytoma

Gemistocytic astrocytomas are the second most common variant in grade II astrocytomas. Smear preparation of gemistocytic astrocytomas are characterised by cells with large, eosinophilc, plump, angulated cytoplasm and eccentric nuclei. In paraffin sections, these tumors display prominent perivascular lymphocyte cuffing.

Grade III Astrocytoma (Anaplastic Astrocytoma)

All low grade astrocytomas eventually progress into grade 3 anaplastic astrocytomas. However, anaplastic astrocytomas can arise denovo also. They usually occur in the fifth decade. Both squash smear and histopathology reveals increased cellularity, cytological atypia and mitosis than low grade astrocytoma. Nuclear alterations such as angulation and dense hyperchromasia are noted.

Grade IV Astrocytoma (Glioblastoma Multiformae)

Glioblastomas (GBM) are the most frequent and most malignant primary brain tumors[26]. They occur typically in the older age group around 5th decade. They arise either denovo (primary) or secondary. On gross inspection, the tumors reveal areas of necrosis. Diffuse infiltration beyond macroscopic borders occur invariably[27]. Smear preparation from glioblastomas exhibit extensive cytoplasmic and nuclear pleomorphism. Patchy areas of necrosis and endovascular proliferation are evident. In paraffin sections, endothelial proliferation and necrosis are the hall mark of glioblastomas.

Two types of necrosis are seen in glioblastoma,

1. Large confluent ischemic necrosis.
2. Multiple, irregularly shaped serpiginous foci of pseudopalisading necrosis.

Ki-67/MIB-1 Labelling Index for Astrocytoma

One of the most important uses of immunohistochemistry in CNS tumors is the MIB-1 labelling index for astrocytoma[28]. This index is a diagnostic and independent prognostic marker in astrocytoma. Ki-67 antigen is a non histone nuclear protein expressed by the actively dividing cells in cell cycle. MIB-1 is the monoclonal antibody

to Ki-67. Hence, as the grade of astrocytoma increases, the propotion of dividing cells in tumor increases. Labelling index or proliferation index is calculated by the ratio of fraction of positive cells to the total number of cells in the slide[29]. While calculating the index, highly cellular area of the slide must be taken.

Labelling index in Low grade astrocytoma <5%.

Labelling index in Anaplastic astrocytoma 5-10%.

Labelling index in Glioblastoma Multiformae >10%.

Pleomorphic Xanthoastrocytoma

Pleomorphic Xanthoastrocytoma is a relatively uncommon glioma accounting for less than 1% of astrocytoma[29]. It occurs most commonly in children and young adults with long standing history of seizures[30]. It occurs equally in both sexes.

Smear preparation of Pleomorphic xanthoastrocytoma is characterised by considerable degree of pleomorphism. The cells are cohesive due to the abundance of stroma. In paraffin sections, the tumor is composed of spindle cells in fascicular array admixed with tumor giant cells with abundant cytoplasm. The cytoplasm appears foamy or lipidized. Despite the disturbing morphology, the tumor tends to present in a benign fashion and complete excision is curative.

Pilocytic Astrocytoma

Pilocytic astrocytoma is a slow growing tumor and comprises of 5-6% of gliomas. It occurs mainly in first two decades of life. It occurs mainly in the midline such as cerebellum, third ventricle and optic chaisma. The piloid cytological features of astrocytes are readily demonstrated in smear preparations. In paraffin sections, pilocytic

astrocytoma is a typically biphasic tumor. Bipolar, fibrillary cells admixed with areas of stellate cells with short processes are seen.

OLIGODENDROGLIAL TUMORS

Oligodendroglioma represent approximately 9.5% of adult gliomas world wide[31]. They occur mainly in adults of the third and fourth decade. They occur mainly in the fronto parietal region and basal ganglia.

On gross inspection, Oligodendrogliomas are soft and gelatinous. Micro calcification is common. In smear preparation, they spread out easily and impart a gritty feel due to calcification.

In smear preparation, the tumor cells have poorly defined cytoplasm and are loosely cohesive in an ill defined eosinophilic matrix. The nuclei of oligodendrocytes are round and slightly lobulated with delicate chromatin pattern. The key feature which is readily apparent in smear preparation is the absence of fibrillary matrix and the abundant delicate microvasculature. In haematoxylin and eosin sections, oligodendroglial tumors show artifactual perinuclear cytoplasmic clearing known as “Fried Egg apperence”. The abundant vascular stroma forms network of thin walled vessels mimicking chicken wire pattern. Proliferation index in oligodendrogliomas is generally less with labelling index of less than 2%[32].

EPENDYMAL TUMORS

Ependymal tumors arise from the ependymal lining of the ventricles and the spinal cord. Ependymomas constitute 2.3% of primary CNS neoplasms world wide[33]. Ependymomas show bimodal age distribution with first peak during early childhood in first five years of life and the second peak in the fourth and fifth decade of life[33]. Ependymomas show definite preponderance for male, with male to female ratio of 1.7:1.

Most of the paediatric ependymomas arise below the tentorium in the posterior fossa. In adults, most of the ependymomas arise in the cervical region of spinal cord. Multiple spinal cord ependymomas are usually associated with Neurofibromatosis type 2.

Ependymoma

Grossly they are soft, papillary with areas of cystic degeneration and calcification. In squash preparation, ependymomas demonstrate tumor cells showing well defined cytoplasm with tapering processes. Most striking in smear preparation is the formation of perivascular pseudo rosettes and nuclear grooving. True ependymal rosettes and epithelial lined tubules are less common.

In haematoxylin and eosin preparations, the tumor cells are round or spindle shaped with granular chromatin and inconspicuous nucleoli. The tumor cell cytoplasmic processes condense around blood vessels to form pseudo rosettes. True ependymal rosettes are less common.

There are four variants of ependymoma including Cellular, tanycytic, papillary and clear cell variants. All variants have same clinical behaviour and prognosis. However they are commonly mistaken for other tumors.

- Cellular ependymoma is mistaken for diffuse glioma.
- Clear cell ependymoma is mistaken for oligodendroglioma.
- Papillary ependymoma is mistaken for metastatic deposits.

Myxopapillary Ependymoma

Myxopapillary ependymomas are restricted to the cauda equina and filum terminale. Rarely they can arise in the cervico thoracic segments of spinal cord. The cell of origin is from the subcutaneous ependymal rests in the cauda equina[34]. They occur in adults in the third to fourth decade of life.

In smear preparations the distinction between chordoma and myxopapillary ependymoma is difficult. In paraffin sections, the tumor is composed of epithelioid cells with exuberant extracellular matrix that is positive for mucin.

EMBRYONAL TUMORS

Embryonal tumors are primitive tumors that occur during the first decade of life. They are clinically aggressive neoplasms. There are certain features which are common to all embryonal tumors.

- All embryonal tumors are hypercellular with increased mitosis.
- Foci of necrosis is invariably present.
- Common propensity for leptomeningeal invasion.
- Metastasis along the CSF pathways.

All these features reflect the aggressive behaviour of embryonal tumors and are designated as WHO grade IV.

MEDULLOBLASTOMA

Medulloblastoma is the most common primitive neuroectodermal tumor. This is the most malignant CNS tumor arising in childhood[35]. They occur in the first decade of life with peak incidence between 5-10 years, with a slight male predominance. Majority of the tumors occur in the cerebellum in relation to the fourth ventricle. The cell of origin of medulloblastoma is the fetal external granular layer of cerebellum.

Grossly, medulloblastoma are soft and friable. Necrosis is invariably present. Desmoplastic medulloblastoma are more firmer due to abundant desmoplastic stroma. The main differential diagnosis to be considered in medulloblastoma of the fourth ventricle is ependymoma. Ependymomas are prone to calcification where as medulloblastomas are not.

In smear preparation, medulloblastoams are hypercellular, composed of cells with scanty cytoplasm and ill defined cell borders. The nuclei are hyperchromatic with irregular membrane often with nuclear moulding.

In haematoxylin and eosin preparations, the tumor cells are arranged in sheets. The tumor cells diffusely infiltrate the cerebellar cortex. Mitosis and necrosis are readily apparent. The characteristic feature is the Homer Wright rosettes composed of tumor cell nuclei dispersed in circular fashion around tangled cytoplasmic processes without central lumen.

Cerebral Neuroblastoma

This is a rare embryonal tumor showing distinct neuronal differentiation. They arise supra tentorially. In squash smears and paraffin sections, the characteristic feature is distinct neuroblastic rosettes known as Homer Wright rosettes. Rosettes consist of tumor cell nuclei disposed in a circular fashion around tangled cytoplasmic processes.

TUMORS OF MENINGES

Meningioma

The meninges cover the brain and spinal cord and protect them. Meningiomas arise from meningothelial cells that are seen in the arachnoid membrane and cap the arachnoid villi. The first description of meningioma dates back to Louis in 1774. He described the characteristic fungal like growth of duramater[36]. It was Cushing who coined the term Meningioma in 1920. Meningiomas occur more commonly in female with female to male ratio of 3:1[14].

Meningiomas can arise at any site where meningothelial cells are present. However the most common sites are in falx, cerebral convexities, sphenoidal ridge, tuberculam sellae and para sellar region. In the spinal region, meningiomas arise most commonly in the thoracic region.

Meningiomas are graded based on the degree of anaplasia and aggressive behaviour.

Grade 1 Meningioma

Grade 1 meningiomas are more common and account for 80% of the meningiomas. They are soft to gelatinous and gritty on squash smear due to psammoma bodies. Microscopically the cells are elongated with oval nuclei, indistinct cytoplasm, pale powdery chromatin and occasional intra nuclear inclusions. Most characteristic are the cellular whorls, and psammoma bodies. The various histological subtypes in grade 1 meningiomas are of no prognostic significance[37].

Grade II Meningioma

They include atypical meningioma, clear cell meningioma and chordoid meningioma. The WHO has the following criteria to label as grade 2 meningioma

1. Containing 4 or more mitosis per 10 high power field

OR

2. Exhibiting atleast 3 of the following,

    a. hypercellularity.

    b. patternless sheet like growth.

    c. small cell component with high nuclear cytoplasmic ratio.

    d. zone of necrosis.

Grade III Meningioma

The WHO defines anaplastic meningiomas as,

1. Containing 20 or more mitosis per 10 high power field.

OR

2. Exhibiting loss of differentiated features resulting in carcinoma, melanoma or sarcoma like appearance.

SCHWANNOMA

Schwannoma is a benign tumor of adult with no sex predilection. Schwannomas arising in CNS occur mainly in the cerebello pontine angle[38]. Bilateral schwannomas are mostly associated with neurofibromatosis.

In squash cytology, they are difficult to spread due to firm rubbery consistency. Both squash cytology and histopathology reveals the characteristic biphasic pattern with cellular Antoni A areas and hypo cellular Antoni B areas. The cellular areas show verocay body formation.

NEUROFIBROMA

Neurofibroma in nervous system presents usually in adult life. It is an unencapsulated benign tumor. Grossly they are well circumscribed and unencapsulated. In squash preparation the cells are elongated with scanty cytoplasm and tapering wavy nuclei. In patients with neurofibromatosis the possibility of malignant change in neurofibroma should be considered[39].

HEMANGIOBLASTOMA

Hemangioblastoma is a tumor of uncertain histiogenesis. It constitutes less than 2.5% of CNS tumors. It occurs sporadically or associated with Von Hippel Lindau syndrome[40]. The age of presentation is the 3rd decade. In syndrome associated cases , it occurs in younger age group.

The squash smear demonstrates two distinct population of cells. Plump lipidized stromal cells admixed with fusiform bland looking endothelial cells. Histopathology typically shows clusters of stromal cells separated by multiple vascular channels. Reticulin staining shows the pericellular pattern.

PRIMARY CNS LYMPHOMA

They occur in elderly male in the 6th and 7th decade of life. The incidence of primary CNS lymphoma is increasing due to increase in incidence of HIV infections[41]. The association of EBV has also been documented.

Smear preparation demonstrates pleomorphic large lymphoid cells in a granular and dirty background. Histopathology resembles that of extra nodal diffuse large B cell lymphoma. The prognosis is very poor.

METASTATIC DEPOSITS

Metastatic deposits are the most common malignant tumors in the CNS. They occur in elderly in the 6th and 7th decade of life. They are more common in male. The most common pattern is adenocarcinoma accounting for 85% of cases. The most common primary sites are the lung, breast, malignant melanoma, renocortical neoplasm and colorectal adenocarcinomas.

In squash cytology, the cellular cohesion is characteristic of metastatic epithelial neoplasm that is foreign to gliomas[42]. Histopathology typically reveals an epithelial neoplasm with brain tumor interface being well delineated.

INFECTIVE LESIONS

Tuberculoma

Tuberculoma is the most common non neoplastic space occupying lesions of brain. It occurs mainly in the cerebellum in children. It is usually localised except in patients with immune compromised status such as HIV.

In squash cytology and histopathology, it presents as caseating granuloma with epithelioid cells, Langhans giant cells and lymphocytes.

Mycoses

Fungal infection of the brain is rare expect in immunocompromised individuals. The incidence is increasing due to HIV. The most common mycosis in CNS is Cryptococcus neoformans followed by Aspergillus[43]. Aspergillus generally invades the CNS from ocular, sino nasal and middle ear infections.

Diagnosis of fungal infections by squash cytology is difficult. Even in histopathology, special stains for fungus are needed to demonstrate where hyphae are few.

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MATERIALS AND METHODS

This is a prospective study. The study was conducted in the Department of Pathology, Coimbatore Medical College, Coimbatore. The study was conducted after obtaining the ethical approval from the Ethical Review Committee of Coimbatore Medical College, Coimbatore. Fifty cases of Neuropathological specimens received between from March 2010 to June 2011 were assessed. The study subjects were patients admitted to the Department of Neurosurgery for space occupying lesions of brain and spinal cord.

Prior Information regarding the name, age, gender, clinical history and radiological findings of each patient were noted. During surgery, upon opening the lesion, small bits of tissue measuring 1-2mm^2 were taken in a fresh state and sent for squash cytology in gauze moistened with saline. The remaining tissues were fixed in 10% formalin and sent later for histopathology.

Preparation of Squash Cytology Smears

Upon receiving the requisition and the specimen, the specimen container and the requisition were checked to ensure that all the information is correct. First, the specimen was inspected grossly. Necrotic and haemorrhagic areas were not used for smearing. Only viable tissue was selected. Then 0.5-1mm^2 of tissue was dissected with a scalpel and placed at one end of a labelled clean glass slide. Then the smear was made by compressing the tissue with the second slide and drawing the second slide quickly across the first slide in order to produce a relatively uniform tissue layer. Care was taken in gauging the appropriate amount of pressure exerted.

The smear preparation was rapidly fixed in isopropyl alcohol for five minutes. The smear was stained with rapid haematoxylin and eosin stain.

Procedure of Rapid Staining

- ❖ Staining in Haematoxylin for 5 minutes.
- ❖ Washing in running tap water till sections turn blue.
- ❖ Differentiation in 1% acid alcohol for 5 seconds.
- ❖ Washed in water.
- ❖ Stained in Eosin for 1 min.
- ❖ Washed in water.
- ❖ Washed in absolute alcohol.
- ❖ Cleared in Xylol.
- ❖ Mounted in DPX.

The time taken to complete the staining procedure was approximately eight to ten minutes.

Staining of Paraffin Embedded Tissue
Procedure

- Sections brought to water.
- Stained in haematoxylin for 15 minutes.
- Sections washed in running tap water.
- Differentiated in 1% acid alcohol – 3 to 4 quick dips.
- Washed in running tap water for 10-20 minutes till sections were blue.
- Stained with eosin for 15 seconds.
- Washed in running tap water.
- Dehydrated in 95% alcohol.
- Absolute alcohol – at least 2 changes.
- Sections in Xylene – 2 changes.
- Mounted in DPX mountant.

The squash smears were reported and correlation with histopathology slides were done. In squash cytology, tumors were also graded and correlated with histopathology tumor grading.

In difficult cases where conclusion could not be made even with histopathology, special stains and immunohistochemistry were used as adjuvants to histopathology. Whenever there was difficulty in grading astrocytomas, tumor grading was done with Ki67/MIB-1 labelling index using immunohistochemistry.

OBSERVATION AND RESULTS

In this study, 50 cases of CNS lesions including tumors and infective lesions were assessed. During the study period from March 2010 to June 2011, the total number of general pathology specimens received at the Department of Pathology, Coimbatore Medical College were 4546. Out of these, 50 cases were from the department of neurosurgery. Hence the prevalence of CNS lesions was 1.1%. (Chart 1)

TABLE 1. DISTRIBUTION OF CNS LESIONS

S.NO	CNS LESIONS	NUMBER OF CASES	PERCENTAGE
1	Astrocytoma	15	30%
2	Meningioma	13	26%
3	Neurofibroma/Schwannoma	6	12%
4	Oligodendroglioma	1	2%
5	Reactive Gliosis	2	4%
6	Infective lesions (Tuberculoma and Aspergillus)	4	8%
7	Medulloblastoma	1	2%
8	Ependymoma	1	2%
9	Hemangioblastoma	2	4%
10	CNS lymphoma	1	2%
11	Metastatic deposits	2	4%
12	Others (Keratocyst and Osteoma)	2	4%
TOTAL NO OF CASES		**50**	**100%**

Out of the 50 cases, 42 cases were CNS tumors accounting for 84% of CNS lesions. The remaining 8 were non neoplastic, including infective lesions like tuberculoma, aspergillus and lesions not specific to CNS such as Osteoma and Keratocyst (Chart 2 & 3).

The most common CNS tumor was astrocytoma and the second common was meningioma.

TABLE 2. AGE DISTRIBUTION OF SPACE OCCUPYING LESIONS OF BRAIN AND SPINAL CORD

S.NO	AGE GROUP	CNS LESIONS
1	0-10 YRS	6
2	11-20 YRS	1
3	21-30 YRS	7
4	31-40 YRS	8
5	41-50 YRS	15
6	51-60 YRS	10
7	>60 YRS	3
TOTAL NO OF CASES		**50**

The age group in this study ranged from 6 years to 70 years with the mean age group of 39.56 years. The maximum incidence of CNS lesions was found to occur in the 4^{th} to 5^{th} decade (Chart 4).

TABLE 3. AGE DISTRIBUTION OF CNS TUMORS

S.NO	AGE GROUP	CNS TUMORS
1	0-10 YRS	5
2	11-20 YRS	0
3	21-30 YRS	5
4	31-40 YRS	5
5	41-50 YRS	14
6	51-60 YRS	10
7	>60 YRS	3
TOTAL NO OF CASES		**42**

The maximum incidence of CNS tumors occured between 41 to 50 years age group. The tumors in the age group of 0 to 10 years were hemangioblastoma, medulloblastoma, pleomorphic xanthoastrocytoma and small cell variant of glioblastoma multiformae.

TABLE 4. AGE DISTRIBUTION OF ASTROCYTOMA

S.NO	AGE	GRADE 2	GRADE 3	GRADE 4
1	<20 YRS	1	-	1
2	21-30 YRS	-	-	-
3	31-40 YRS	1	2	-
4	41-50 YRS	1	-	3
5	51-60 YRS	1	-	4
6	>60 YRS	1	-	-

Grade 4 Glioblastoma multiformae were found to occur in the 4th and 5th decade of life. Only one case of small cell variant of glioblastoma multiformae occured at 6 years of age. Grade 3 astrocytoma were seen in the 3rd to 4th decade of life. Grade 2 astrocytomas occurred in all age groups with wide age distribution (Chart 5).

TABLE 5. AGE DISTRIBUTION OF MENINGIOMA

S.NO	AGE GROUP	MENINGIOMA
1.	<20 YRS	-
2.	21-30 YRS	4
3.	31-40 YRS	1
4.	41-50 YRS	5
5.	51-60 YRS	3
6.	>60 YRS	-
TOTAL NO OF CASES		**13**

Meningiomas were found to occur in the 4th to 5th decade and the second most common age group were the 2nd to 3rd decade (Chart 6).

TABLE 6. GENDER DISTRIBUTION OF CNS LESIONS

In this study of 50 cases, 26 patients were men and 24 patients were women.

S. NO	CNS LESIONS	MALE	FEMALE
1.	Astrocytoma	10	5
2.	Meningioma	4	9
3.	Neurofibroma/Schwannoma	1	5
4.	Oligodendroglioma	0	1
5.	Medulloblastoma	1	0
6.	Ependymoma	1	0
7.	Hemangioblastoma	0	2
8.	CNS lymphoma	1	0
9.	Metastatic deposits	2	0
10.	Others	6	2
TOTAL NO OF CASES		**26**	**24**

In our study, Astrocytomas were found to be more common in men and Meningiomas, more common in women (Chart 7).

TABLE 7. DISTRIBUTION OF HISTOLOGICAL GRADE OF ASTROCYTOMA

S. NO	ASTROCYTOMA	NO OF CASES
1.	Grade 1	0
2.	Grade 2	5
3.	Grade 3	2
4.	Grade 4	8
TOTAL NO OF CASES		**15**

In Astrocytomas, Grade 4 astrocytomas were the most common histological type followed by grade 2 astrocytomas.

TABLE 8. DISTRIBUTION OF SITE OF INVOLVEMENT IN CNS TUMORS

Among the 42 tumors, 36 occurred in the central nervous system and 6 occurred in the peripheral nervous system. Out of 36 tumors in CNS, middle cranial fossa was found to be the predominant site (Chart 8).

S.NO	SITE	NO OF CASES
1.	Anterior cranial fossa	7
2.	Middle cranial fossa	22
3.	Posterior cranial fossa	7
TOTAL NO OF CASES		**36**

TABLE 9. DISTRIBUTION OF SITE OF INVOLVEMENT IN ASTROCYTOMA

S.NO	SITE	NO OF CASES
1.	Anterior cranial fossa	3
2.	Middle cranial fossa	11
3.	Posterior cranial fossa	1
TOTAL NO OF CASES		**15**

In astrocytomas, the most common site of tumor was found to be the middle cranial fossa.

TABLE 10. CORRELATION OF RADIOLOGICAL DIAGNOSIS WITH HISTOPATHOLOGY

Out of 50 cases, radiological investigations provided the correct diagnosis in 30 cases with the diagnostic accuracy of 60% (Chart 9).

S.NO	CNS LESIONS	CORRELATION WITH HPE		DIAGNOSTIC ACCURACY
		CORRELATION	NO CORRELATION	
1.	Astrocytoma	9	6	60%
2.	Meningioma	9	4	69.23%
3.	Neurofibroma/Schwannoma	6	-	100%
4.	Oligodendroglioma	1	-	100%
5.	Medulloblastoma	1	-	100%
6.	Ependymoma	-	1	0%
7.	Hemangioblastoma	-	2	0%
8.	CNS lymphoma	1	-	100%
9.	Metastatic deposits	-	2	0%
10.	Others	3	5	37.5%
TOTAL NO OF CASES		**30**	**20**	**60%**

The diagnostic accuracy by radiology was 100% for Neurofibroma / Schwannoma, Oligodendroglioma, Medulloblastoma and CNS lymphoma. In Astrocytoma, the accuracy was 60% and in Meningioma it was found to be 69.23%.

TABLE 11. CORRELATION OF SQUASH CYTOLOGY WITH HISTOPATHOLOGY

Out of 50 cases, correct correlation was obtained in 37 cases with diagnostic accuracy of 74% and in the remaining 13 cases, diagnosis could not be precisely established with squash cytology (Chart 10).

S.NO	CNS LESIONS	CORRELATION WITH HPE		DIAGNOSTIC ACCURACY
		CORRELATION	NO CORRELATION	
1.	Astrocytoma	14	1	93.33%
2.	Meningioma	11	2	84.62%
3.	Neurofibroma/Schwannoma	5	1	83.33%
4.	Oligodendroglioma	-	1	0%
5.	Medulloblastoma	1	-	100%
6.	Ependymoma	1	0	100%
7.	Hemangioblastoma	1	1	50%
8.	CNS lymphoma	1	-	100%
9.	Metastatic deposits	1	1	50%
10.	Others	2	6	25%
TOTAL NO OF CASES		**37**	**13**	**74%**

TABLE 12. CORRELATION OF SQUASH CYTOLOGY WITH HISTOPATHOLOGY IN THE GRADING OF ASTROCYTOMA

S.NO	GRADE	NO OF CASES	NO OF CASES
		CORRECT GRADE	WRONG GRADE
1.	II	4	1
2.	III	2	-
3.	IV	5	3
TOTAL NO OF CASES		**11**	**4**

The diagnostic accuracy of squash cytology in astrocytoma was 93.33%. However when predicting the grade of astrocytomas, the diagnostic accuracy decreased to 73.33%. Correct grading was possible in 11 cases out of 15 (Chart 12).

TABLE 13. DIAGNOSTIC ACCURACY OF SQUASH CYTOLOGY IN GRADING ASTROCYTOMA

S.NO	GRADE	DIAGNOSTIC ACCURACY
1.	Grade 2	80%
2.	Grade 3	100%
3.	Grade 4	62.5%

Grade 3 astrocytoma had diagnostic accuracy of 100% followed by grade 2 which had diagnostic accuracy of 80%. In glioblastoma multiformae correct grading was possible only in 5 cases out of 8 with diagnostic accuracy of 62.5% (Chart 13).

TABLE 14. SENSITIVITY AND SPECIFICITY OF SQUASH CYTOLOGY IN DETECTING CNS TUMORS

		HISTOPATHOLOGY	
		CNS TUMOR PRESENT	**CNS TUMOR ABSENT**
SQUASHCYTOLOGY	**CNS TUMOR PRESENT**	41 (a)	2 (b)
	CNS TUMOR ABSENT	1 (c)	6 (d)

The Sensitivity of squash cytology in detecting CNS tumors in the present study was 97.62% and the Specificity was 75%.

The positive predictive value in detecting CNS tumors was 95.35% and the negative predictive value was 85.71%.

The percentage of false positive cases in the study was 25% and the percentage of false negative cases was 2.38%.

LIST OF CHARTS

CHART 1. COMPARATIVE PREVALENCE OF CNS LESIONS

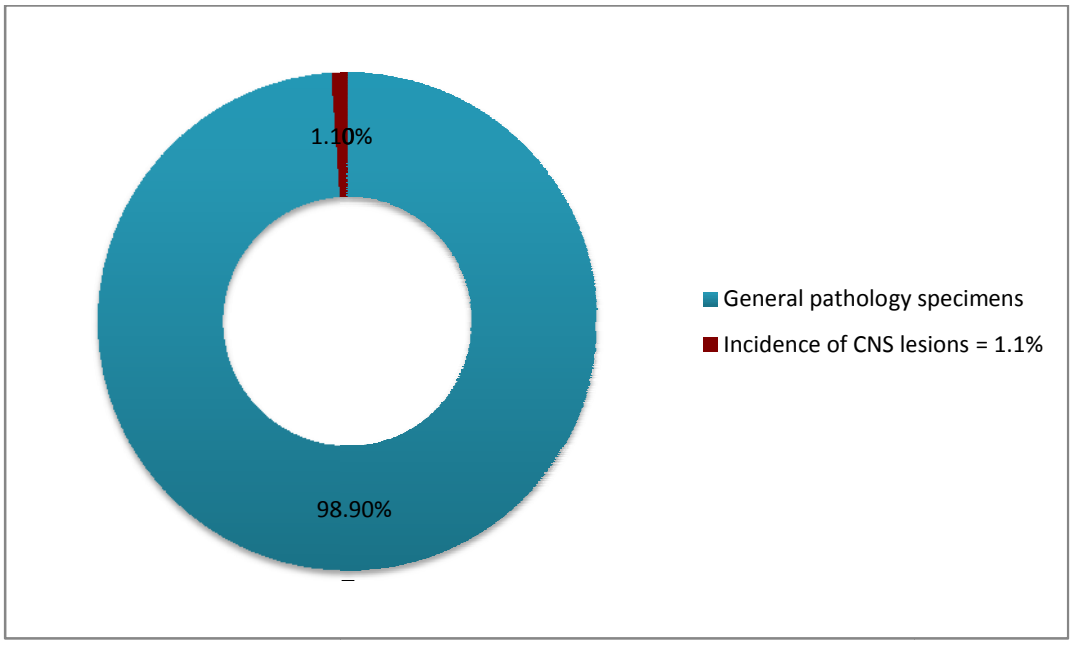

Chart 1.The general prevalence of CNS lesions reported at department of pathology, Coimbatore medical college hospital was 1.1%.

CHART 2. INCIDENCE OF BRAIN TUMORS

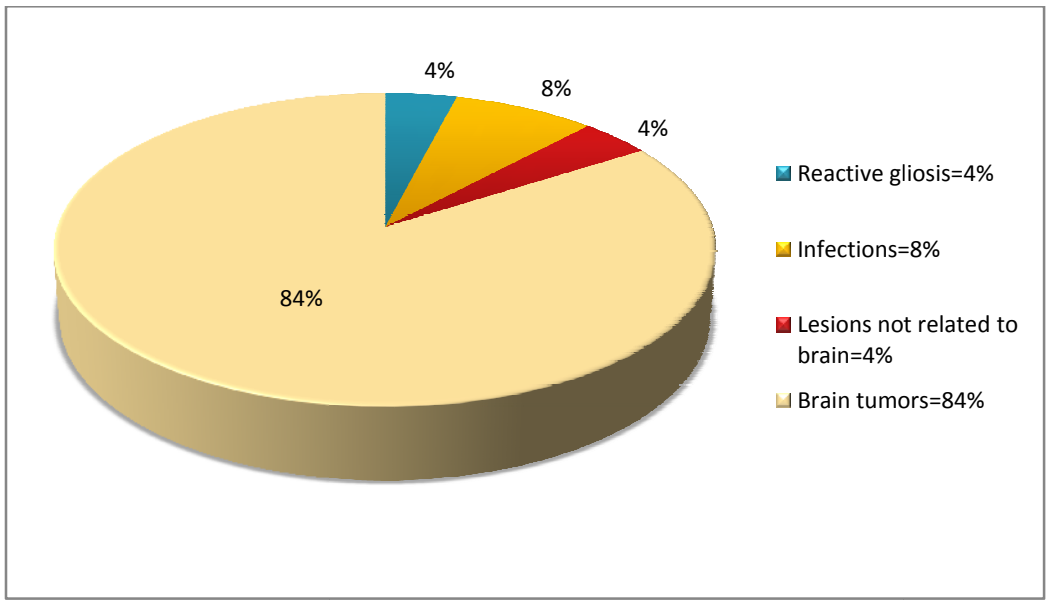

Chart 2. Among the CNS lesions, the incidence of brain tumors were 84%.

CHART 3. DISTRIBUTION OF CNS LESIONS

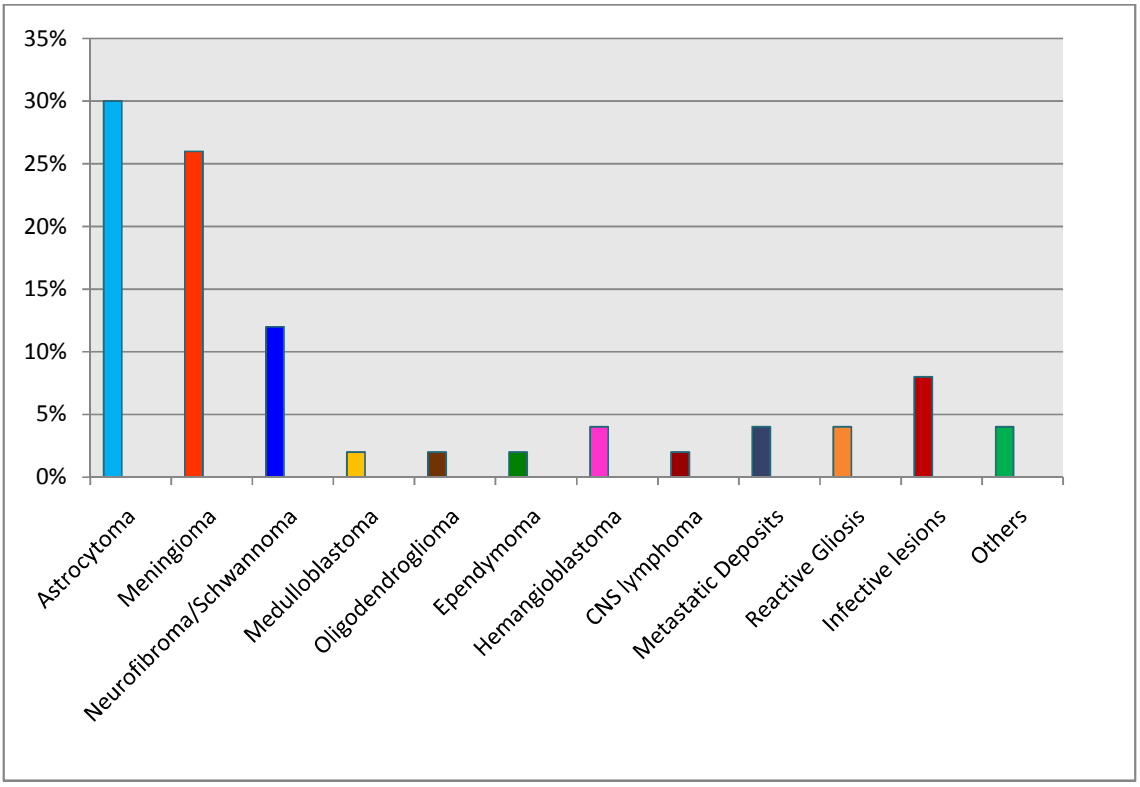

Chart 3. The most common CNS tumor in the present study was astrocytoma followed by meningioma.

CHART 4. AGE DISTRIBUTION OF SPACE OCCUPYING LESIONS OF BRAIN AND SPINAL CORD

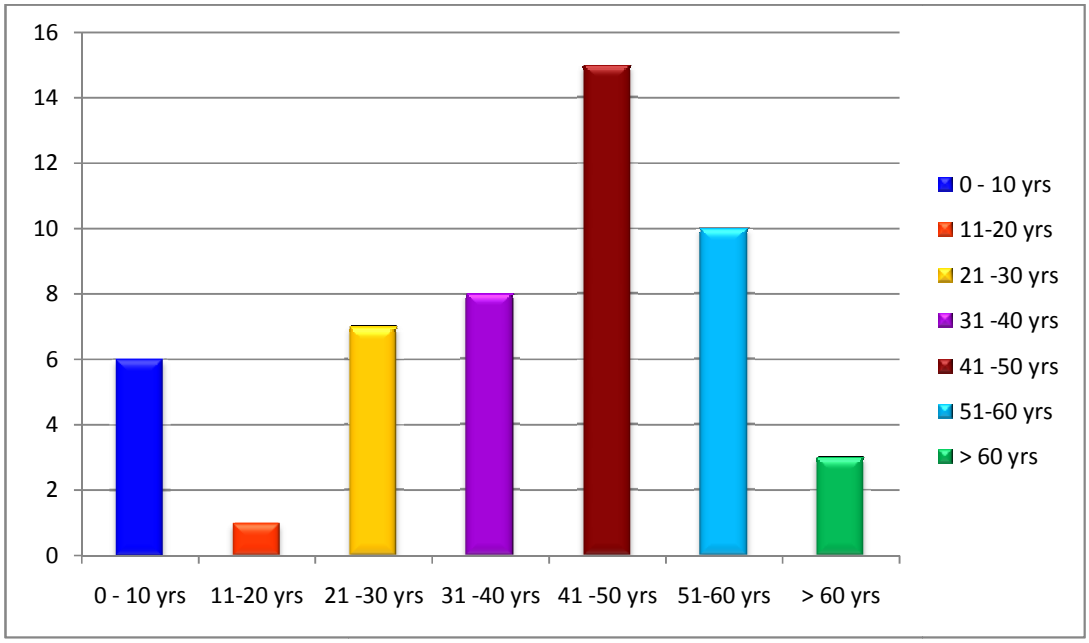

Chart 4. The most common age group affected with CNS lesions was between 41 to 50 years of life.

CHART 5. AGE DISTRIBUTION OF ASTROCYTOMA

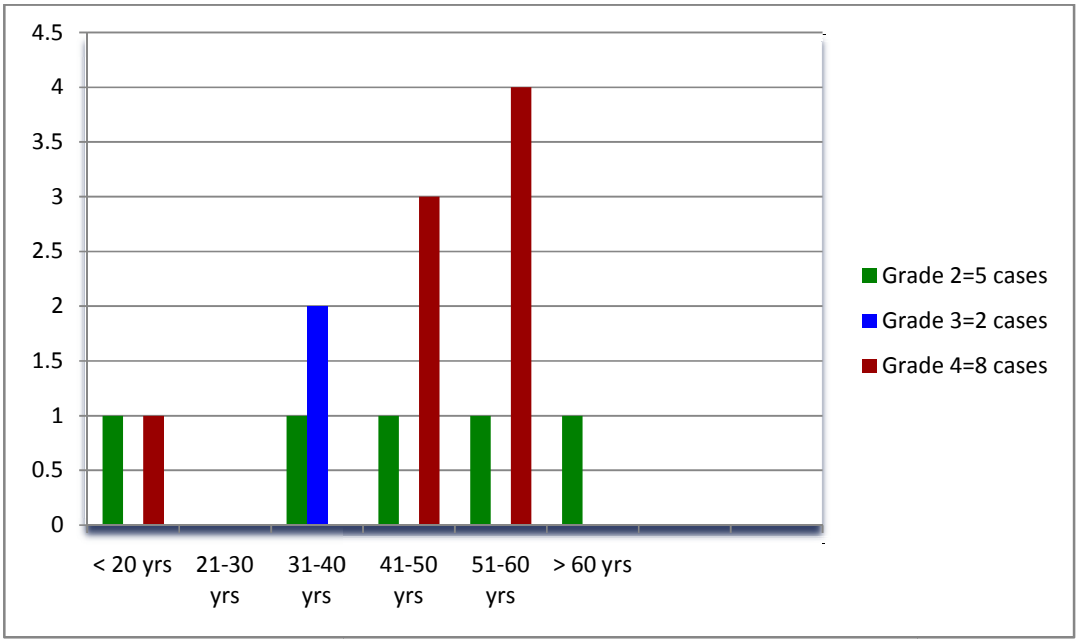

CHART 6. AGE DISTRIBUTION OF MENINGIOMA

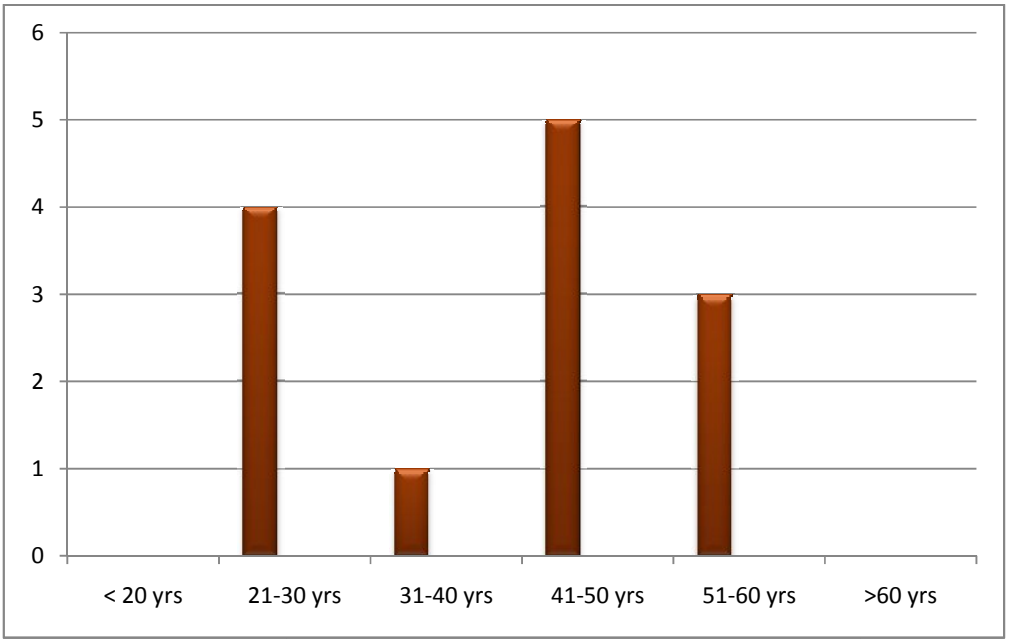

CHART 7. GENDER DISTRIBUTION OF CNS LESIONS

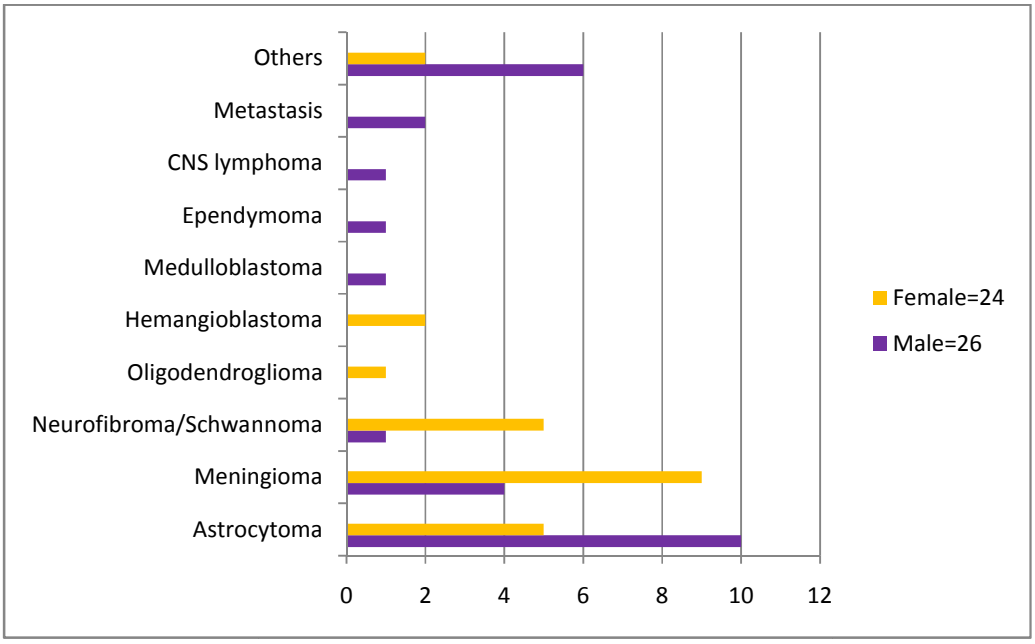

CHART 8. DISTRIBUTION OF SITE OF INVOLVEMENT IN CNS TUMORS

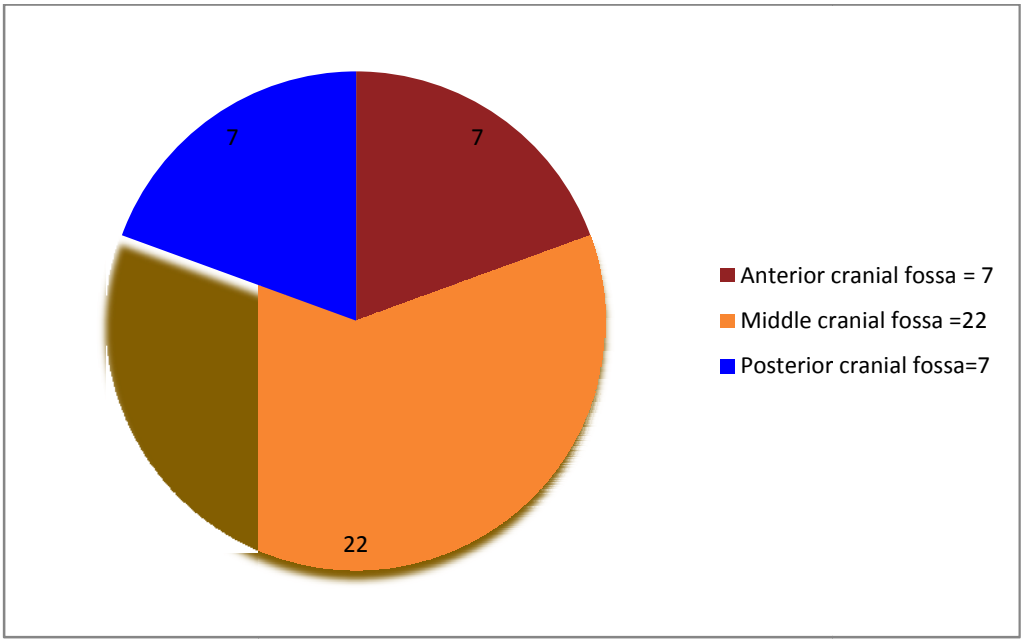

Chart 8. The most common site involved in the study was middle cranial fossa.

CHART 9. CORRELATION OF RADIOLOGICAL DIAGNOSIS WITH HISTOPATHOLOGY

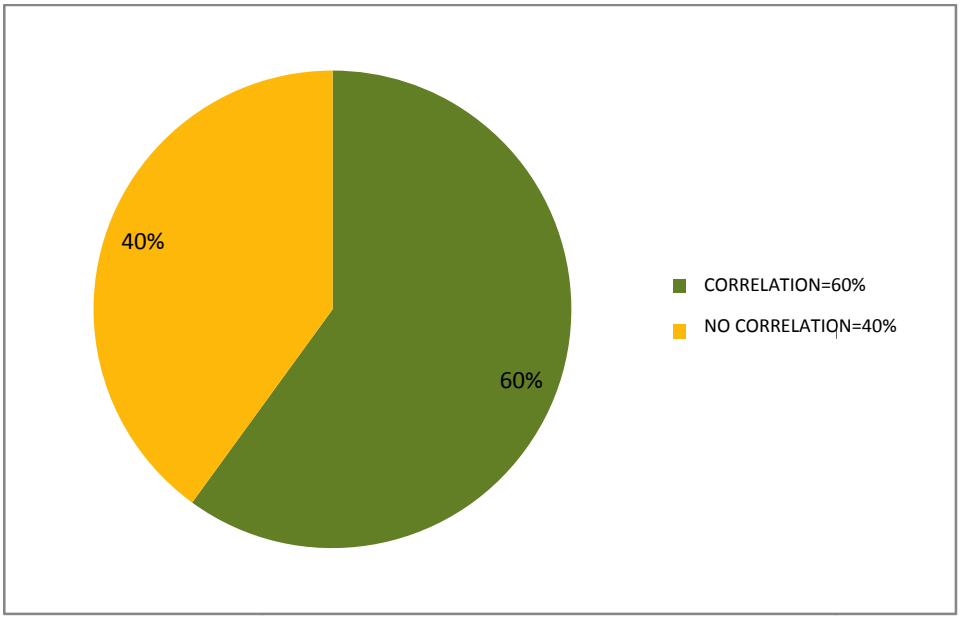

Chart 9. The diagnostic accuracy of radiology in CNS lesions was found to be 60%.

CHART 10. CORRELATION OF SQUASH CYTOLOGY WITH HISTOPATHOLOGY

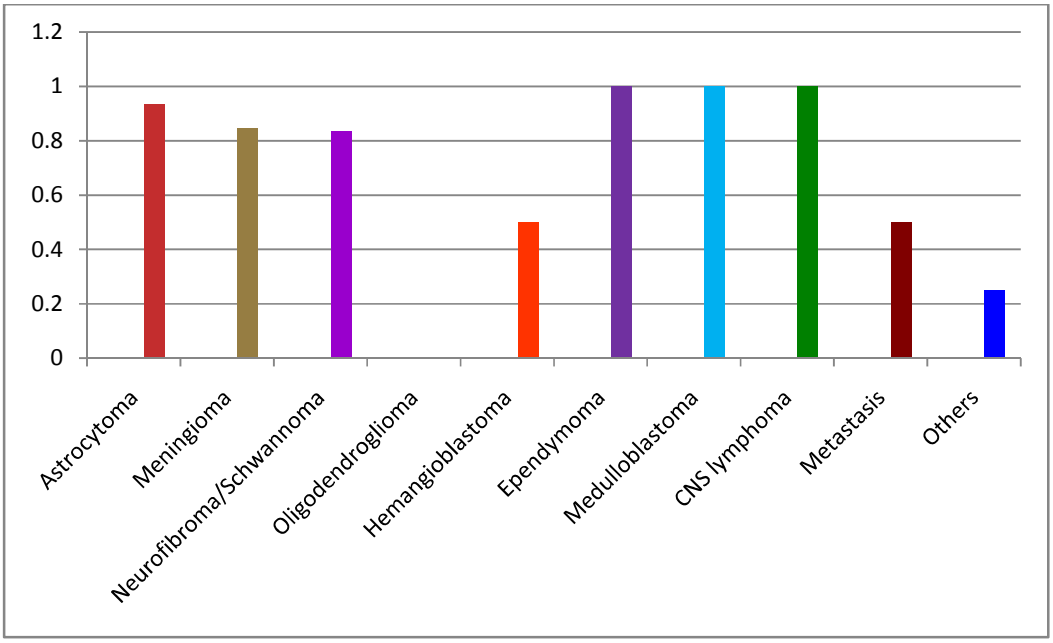

Chart 10. The diagnostic accuracy of squash cytology in detecting CNS lesions was found to be 74%.

CHART 11. COMPARISON OF RADIOLOGICAL DIAGNOSTIC ACCURACY WITH SQUASH CYTOLOGY

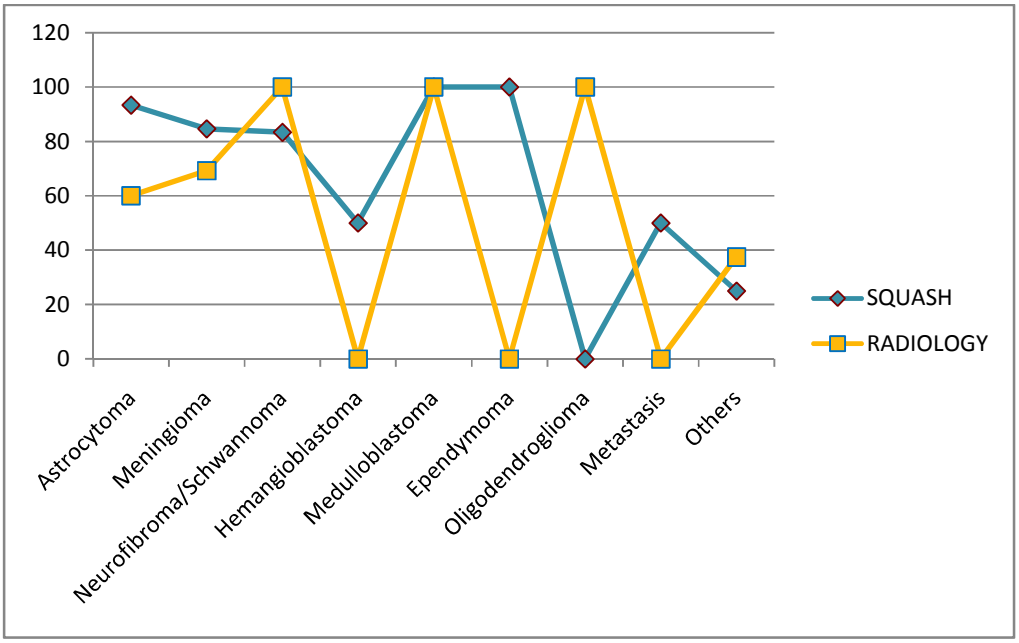

CHART 12. CORRELATION OF SQUASH CYTOLOGY WITH HISTOPATHOLOGY IN GRADING ASTROCYTOMA

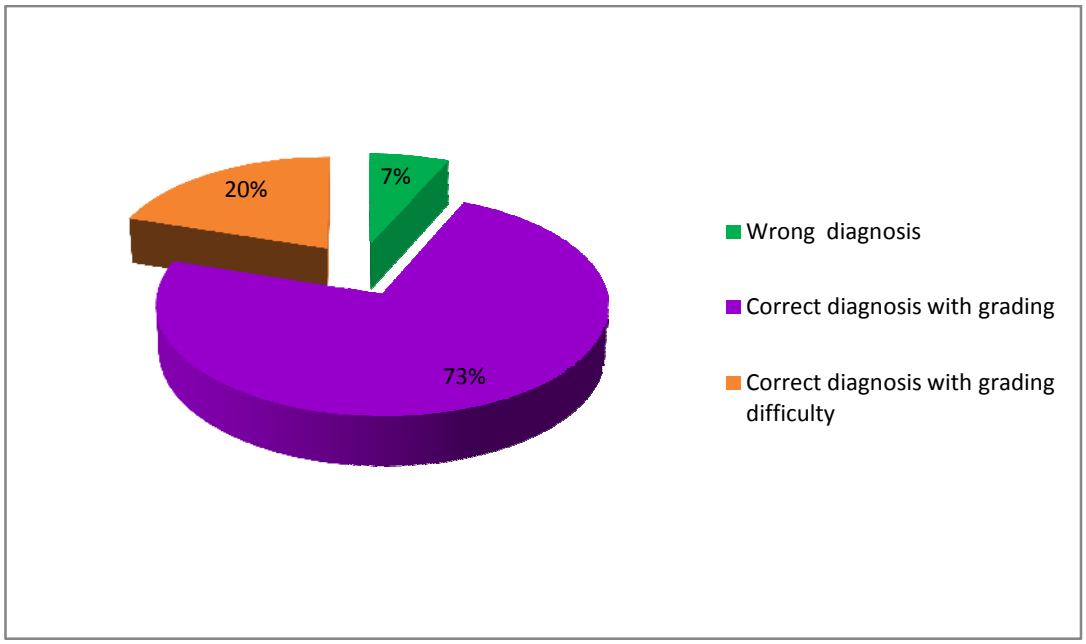

CHART 13. DIAGNOSTIC ACCURACY OF SQUASH CYTOLOGY IN GRADING ASTROCYTOMA

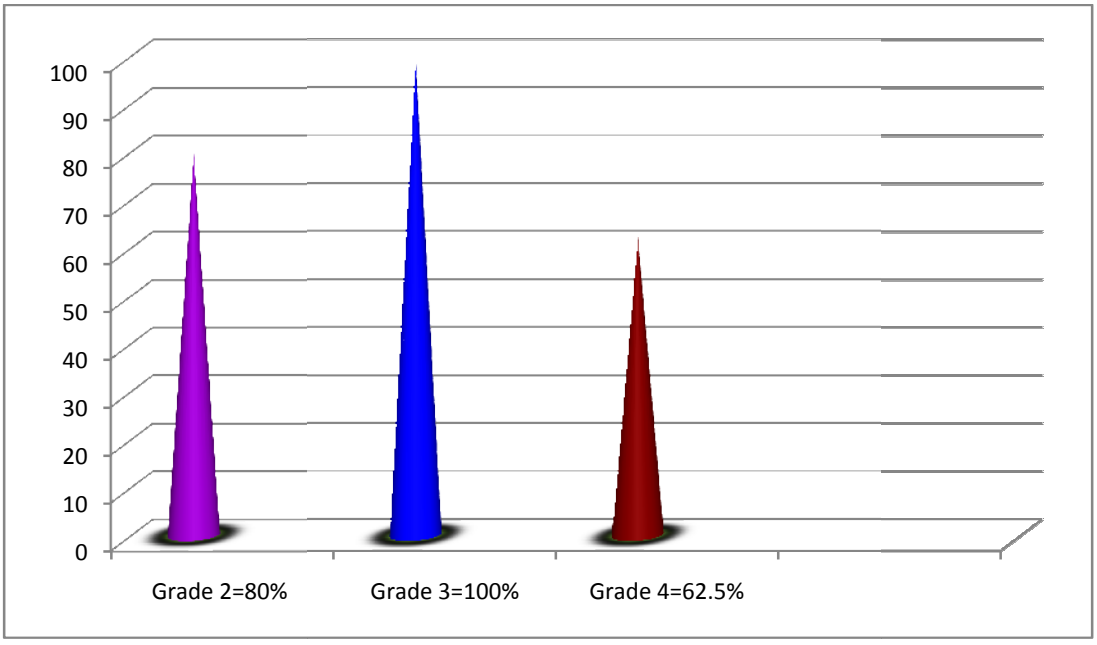

FIGURE 1: SQUASH CYTOLOGY IN GRADE 2 ASTROCYTOMA

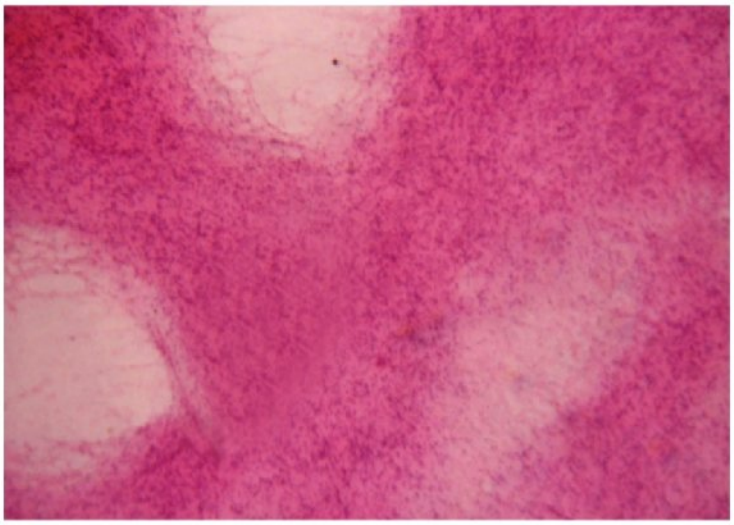

Figure 1 : Low grade astrocytoma showing cells with delicate processes and eosinophilic cytoplasm in a fibrillary background (40X).

FIGURE 2: SQUASH CYTOLOGY IN GRADE 3 ASTROCYTOMA

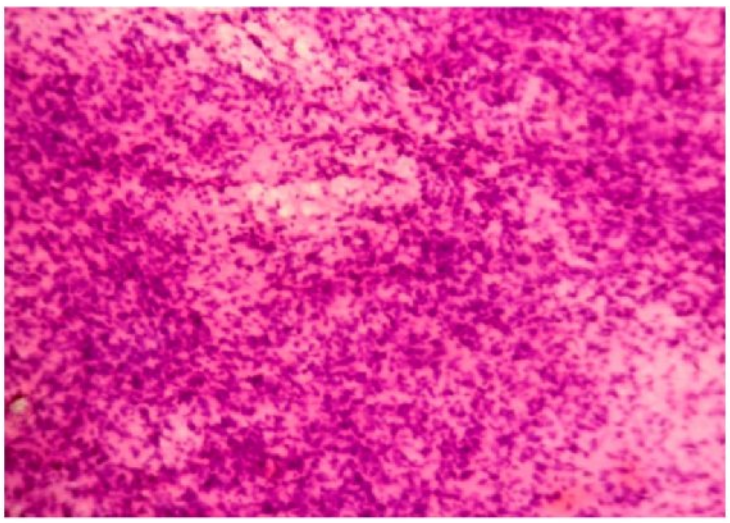

Figure 2 : Grade 3 astrocytoma showing increased cellularity with increased nuclear cytoplasmic ratio (100X).

FIGURE 3: HISTOPATHOLOGY IN GRADE 2 ASTROCYTOMA

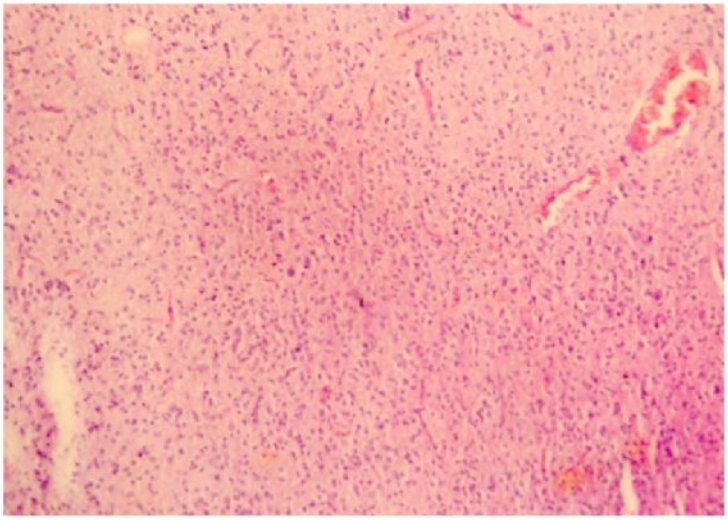

Figure 3 : Grade 2 astrocytoma showing patternless array of neoplastic astrocytes in an eosinophilic fibrillary background (100X).

FIGURE 4: HISTOPATHOLOGY IN GRADE 3 ASTROCYTOMA

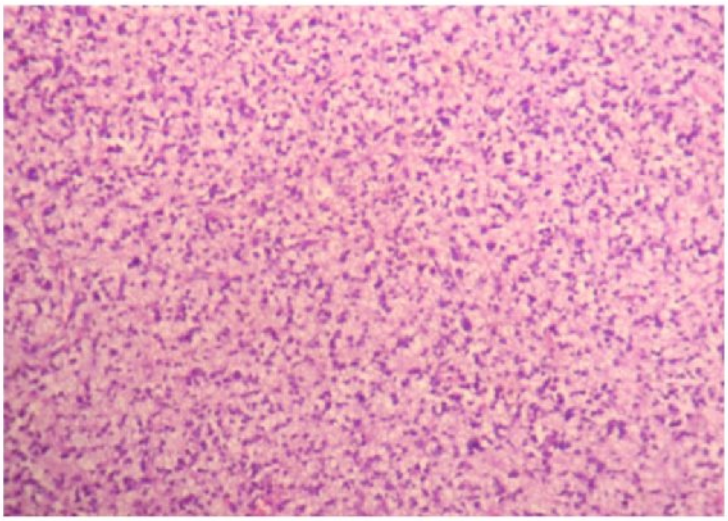

Figure 4 : Grade 3 astrocytoma showing hypercellularity, diffuse anaplasia and wide spread nuclear pleomorphism (100X).

FIGURE 5: SQUASH CYTOLOGY IN GRADE 4 ASTROCYTOMA

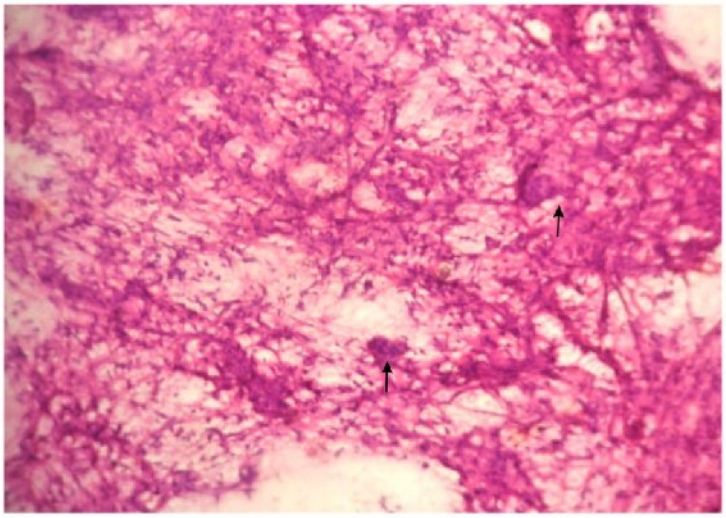

Figure 5: Glioblastoma showing pleomorphic astrocytes and endothelial proliferation(arrow) (100X).

FIGURE 6: HISTOPATHOLOGY IN GLIOBLASTOMA MULTIFORMAE

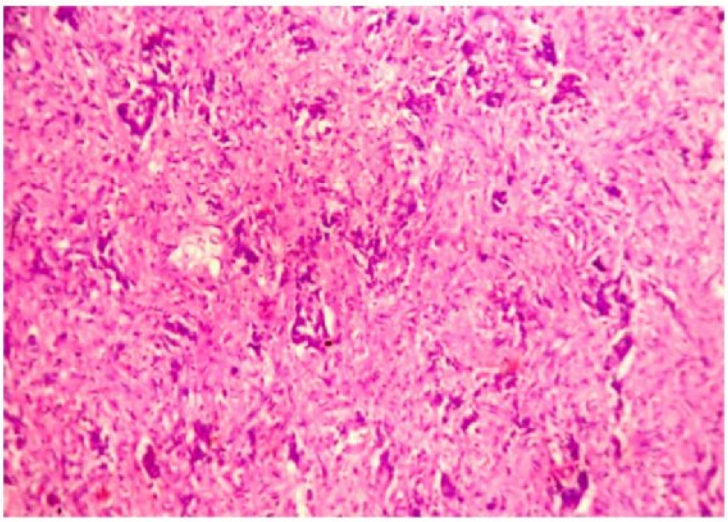

Figure 6: Grade 4 astrocytoma showing endovascular proliferation (100X).

FIGURE 7: Ki67/MIB1 STAINING PATTERN IN GLIOBLASTOMA

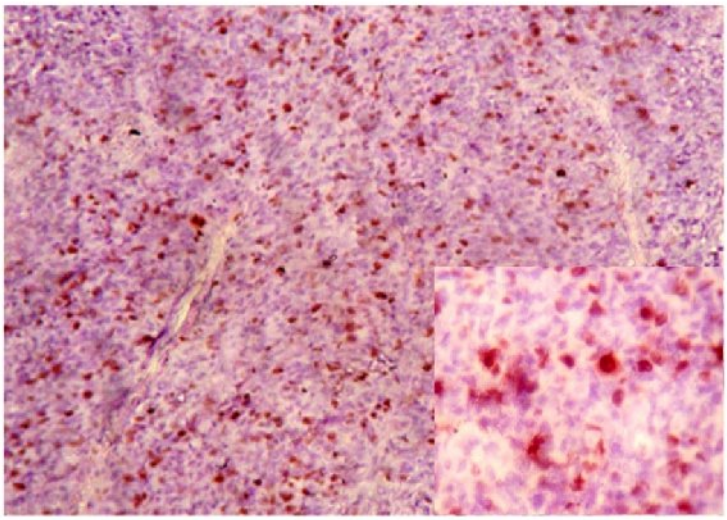

Figure 7: Ki67/MIB1 - A high proliferation index of darkely stained nuclei is shown in this immuno histochemical staining of glioblastoma 100X (IHC). Inset showing high power view 400X (IHC).

FIGURE 8: SQUASH CYTOLOGY IN PSAMMOMATOUS MENINGIOMA

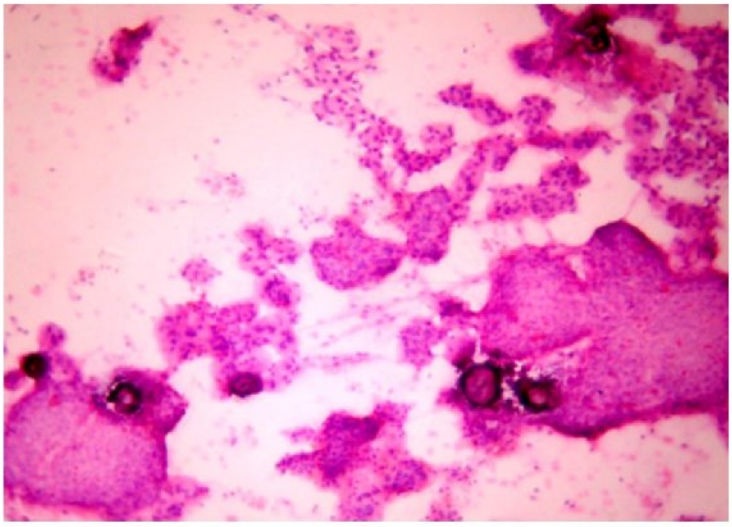

Figure 8: Psammomatous meningioma showing intact cellular whorls and psammoma bodies (100X).

FIGURE 9: HISTOPATHOLOGY IN PSAMMOMATOUS MENINGIOMA

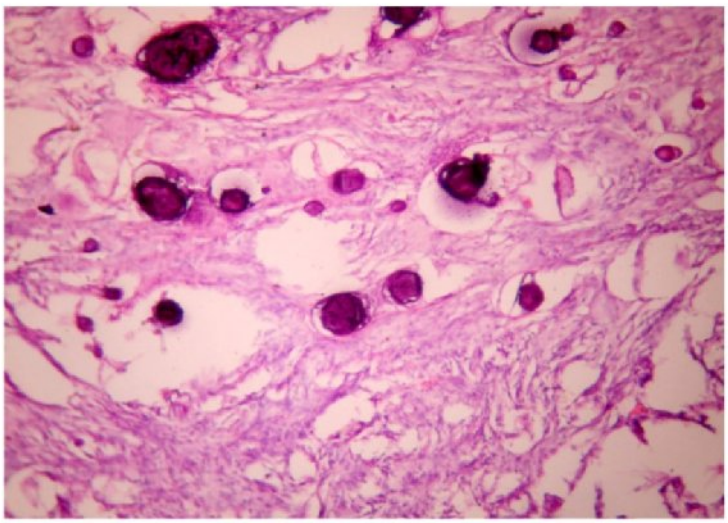

Figure 9: Meningothelial whorls and psammoma bodies (100X).

FIGURE 10: SQUASH CYTOLOGY IN TRANSITIONAL MENINGIOMA

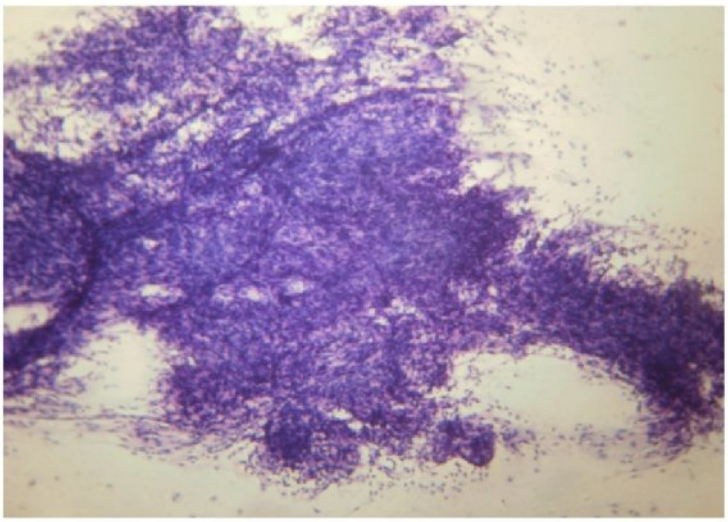

Figure 10: Transitional meningioma showing admixture of meningothelial and fibrous regions (100X).

FIGURE 11: HISTOPATHOLOGY IN TRANSITIONAL MENINGIOMA

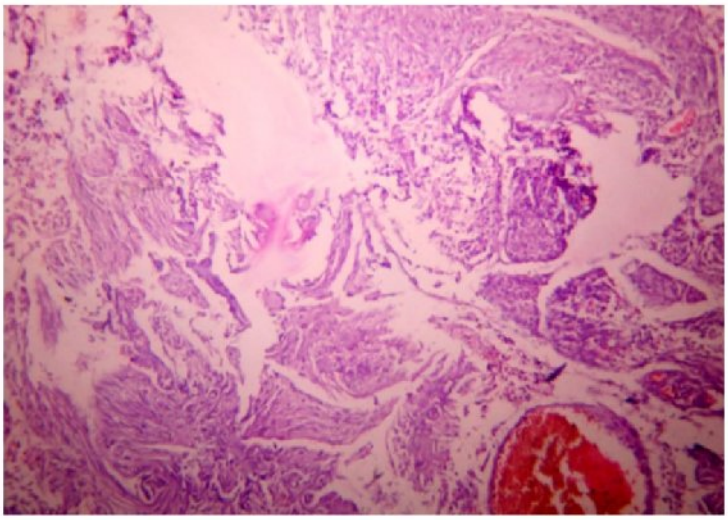

Figure 11: Transitional meningioma displaying features of both meningothelial and fibroblastic meningiomas (100X).

FIGURE 12: SQUASH CYTOLOGY IN SCHWANNOMA

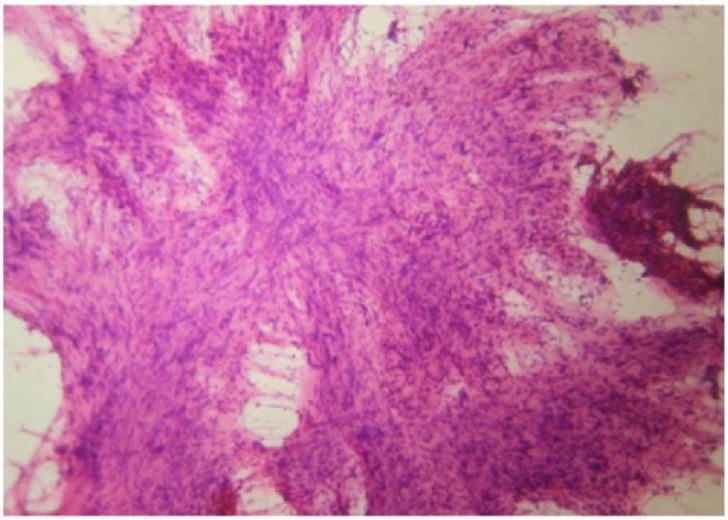

Figure 12: Schwannoma showing fascicles of tumor composed of cohesive antoni A areas (100X).

FIGURE 13: HISTOPATHOLOGY IN SCHWANNOMA

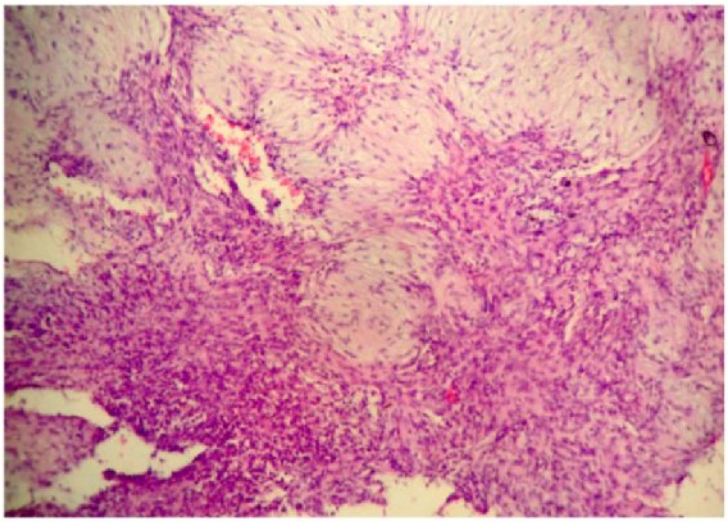

Figure 13: Schwannoma composed of swirls of compact antoni A and loose antoni B areas (100X).

FIGURE 14: S-100 STAINING PATTERN IN SCHWANNOMA

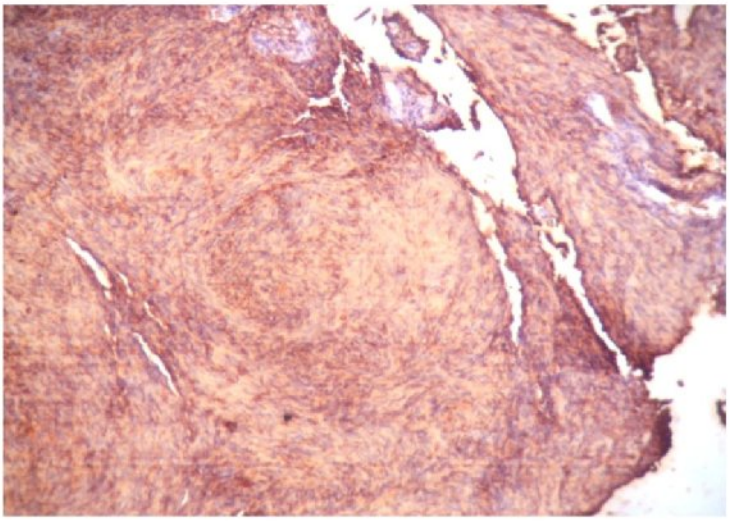

Figure 14: Schwannoma showing strong and diffuse S100 positivity 100X(IHC).

FIGURE 15: SQUASH CYTOLOGY METASTATIC ADENO CARCINOMATOUS DEPOSITS

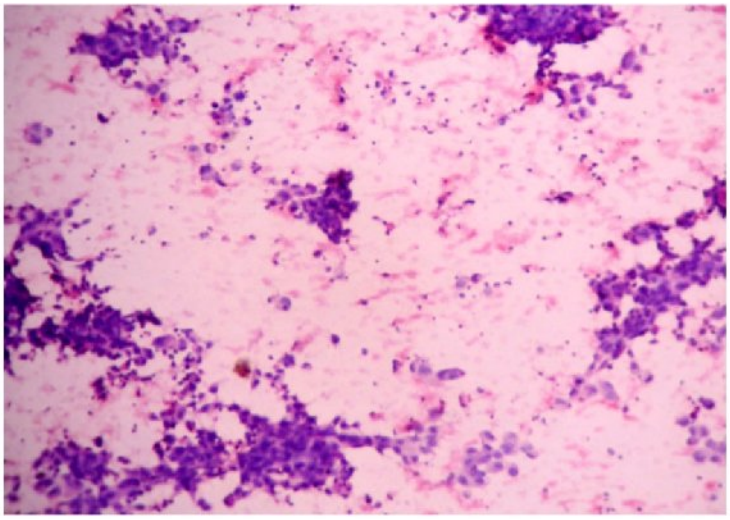

Figure 15: Cohesive clusters of large cells with abundant cytoplasm arranged in vague glandular pattern (100X).

FIGURE 16: HISTOPATHOLOGY IN POORLY DIFFERENTIATED CARCINOMATOUS DEPOSITS

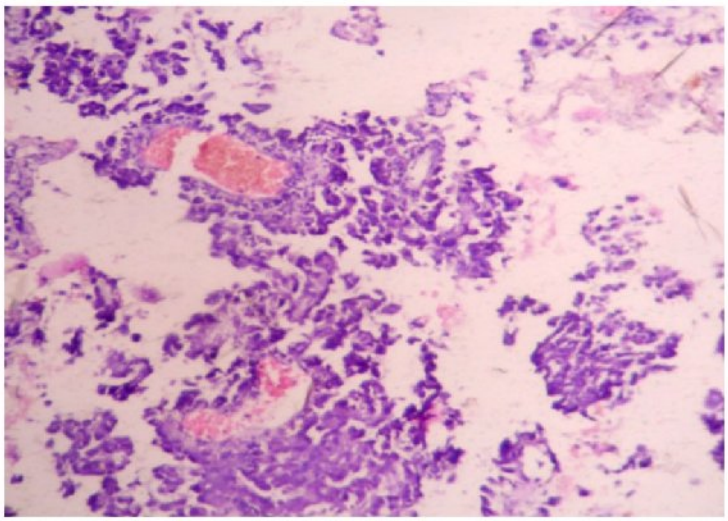

Figure16: Highly anaplatic tumor cells arranged in glandular pattern around blood vessels (100X).

FIGURE 17: SQUASH CYTOLOGY IN OLIGODENDROGLIOMA

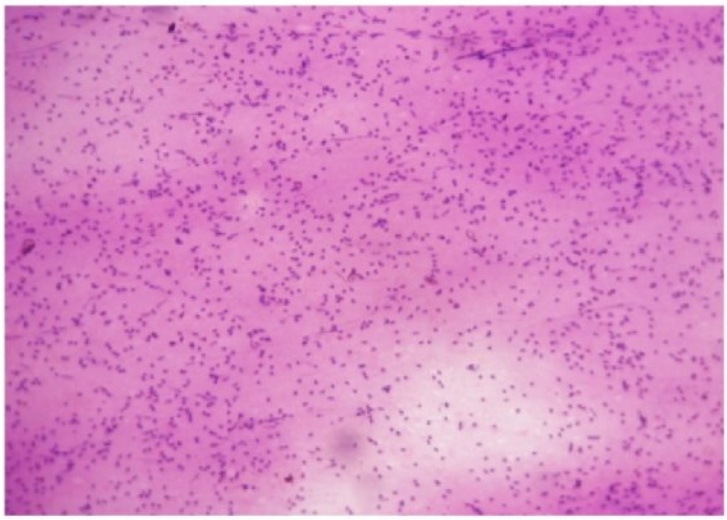

Figure 17: Oligodendroglioma showing even spread of cells with rounded nuclei without perinuclear halo (100X).

FIGURE 18: HISTOPATHOLOGY IN OLIGODENDROGLIOMA

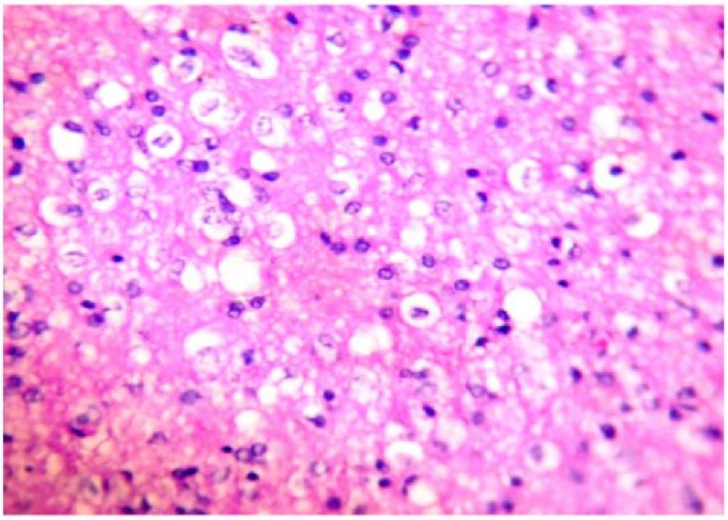

Figure 18: Oligodendroglioma with peri nuclear halo showing "Fried Egg appearance" (400X).

FIGURE 19: SQUASH CYTOLOGY IN HEMANGIOBLASTOMA

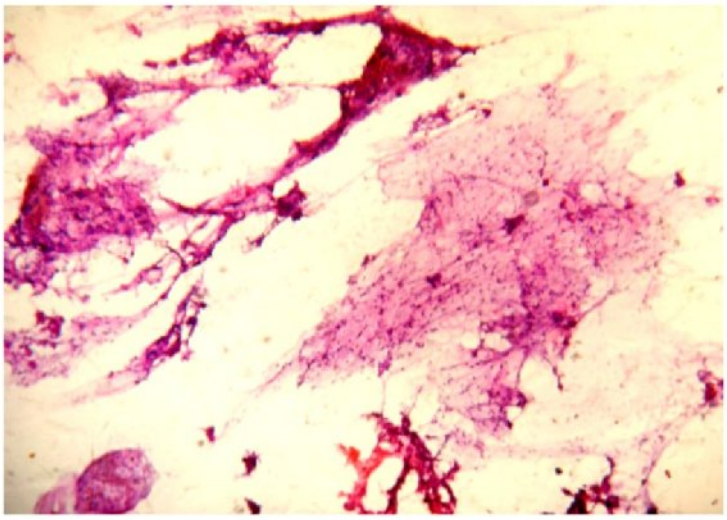

Figure 19: Thickly smeared tissue enclosing irregular vascular spaces (40X).

FIGURE 20: HISTOPATHOLOGY IN HEMANGIOBLASTOMA

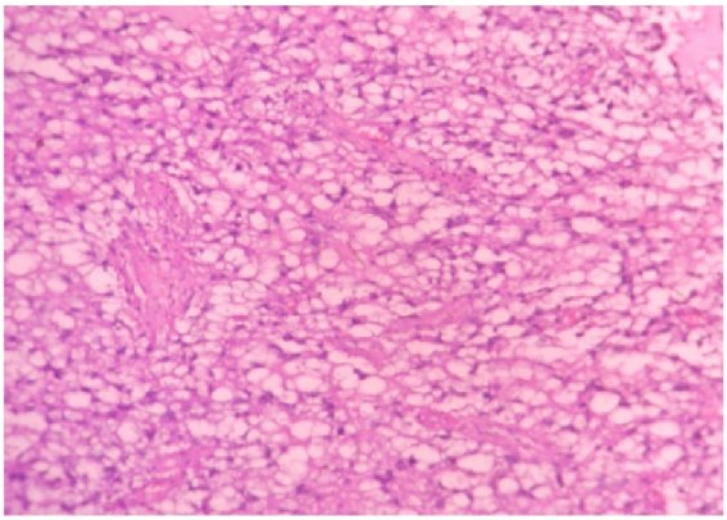

Figure 20: Sheets of neoplastic stromal cells intersected by numerous capillary channels (100X).

FIGURE 21: RETICULIN STAINING IN HEMANGIOBLASTOMA

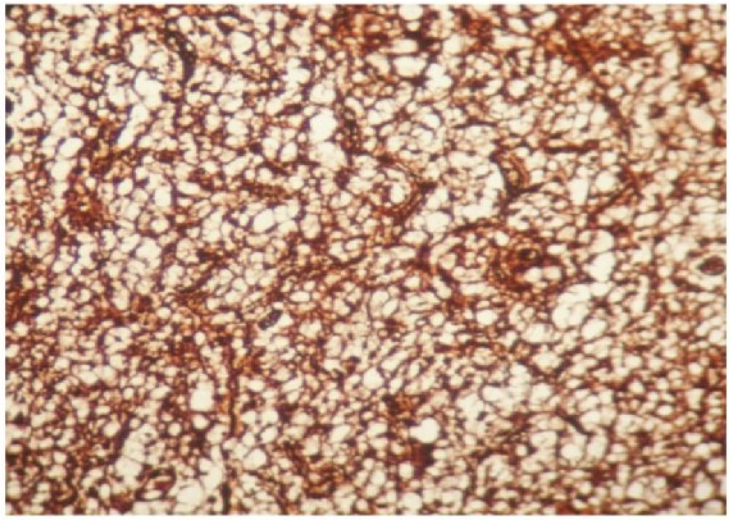

Figure 21: Hemangioblastoma showing dense pericellular pattern (100X - Reticulin).

FIGURE 22: CD34 IMMUNO STAINING IN HEMANGIOBLASTOMA

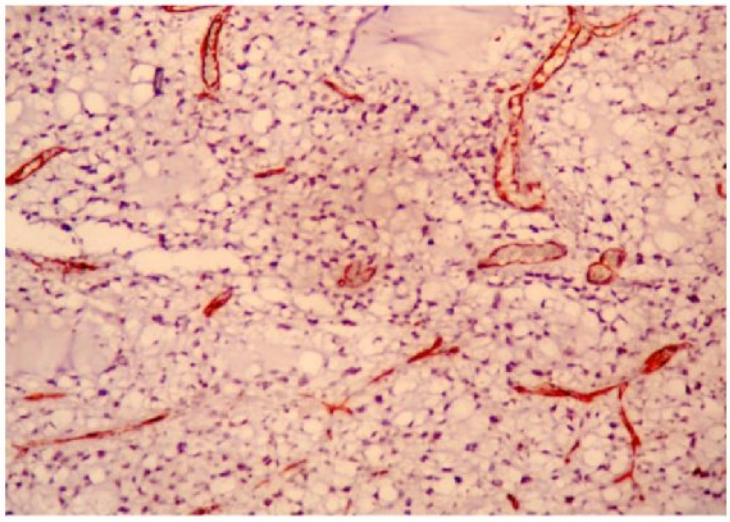

Figure 22: CD34 showing cytoplasmic positivity in the endothelial cells 100X(IHC).

FIGURE 23: SQUASH CYTOLOGY IN PLEOMORPHIC XANTHOASTROCYTOMA

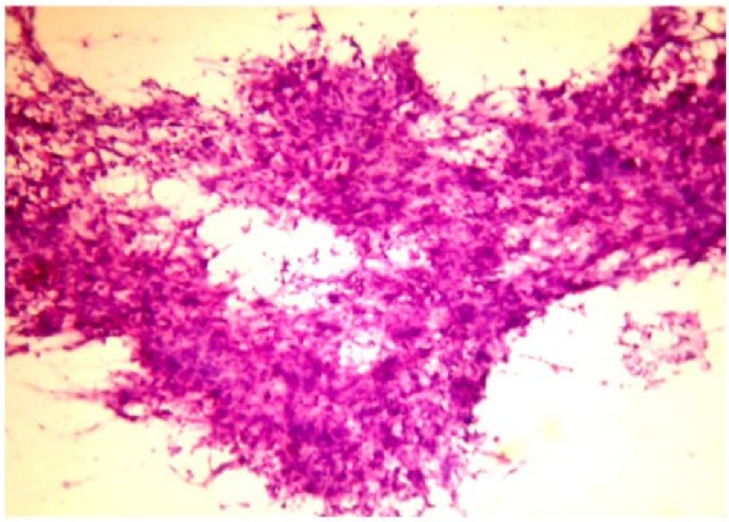

Figure 23: Showing bizarre nature of cells with multinucleated giant cells (100X).

FIGURE 24: HISTOPATHOLOGY IN PLEOMORPHIC XANTHOASTROCYTOMA

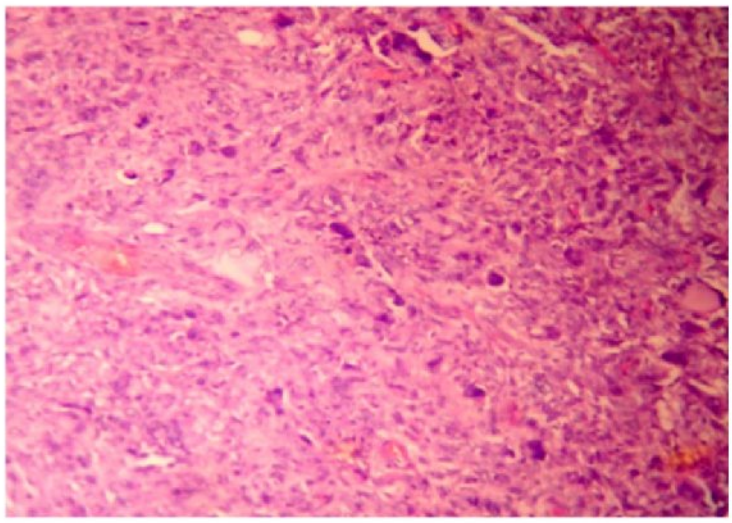

Figure 24: Many multinucleated giant cells and bizarre cells in pleomorphic xanthoastrocytoma (100X).

FIGURE 25: SQUASH CYTOLOGY IN EPENDYMOMA

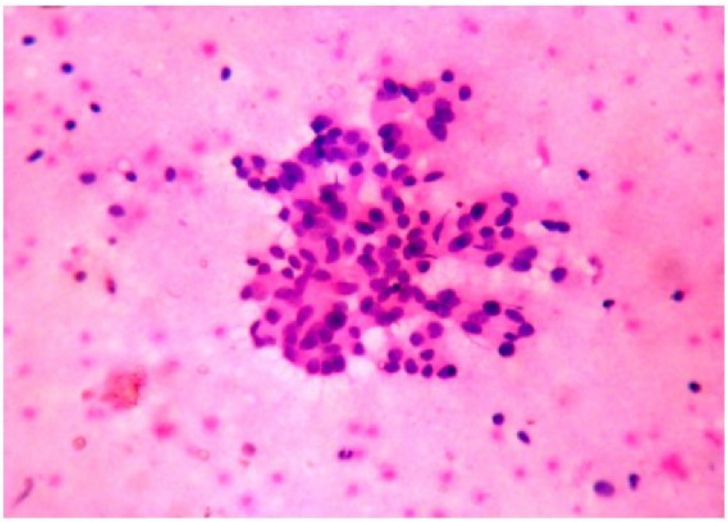

Figure 25: Ependymoma showing papillary pattern and rosette like structures (400X).

FIGURE 26: HISTOPATHOLOGY IN MYXOPAPILLARY EPENDYMOMA

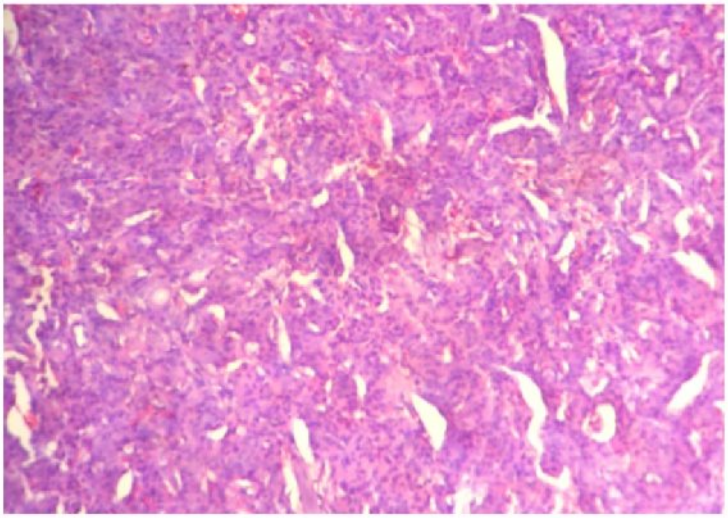

Figure 26: showing papillary structures surrounded by epithelial like layer of ependymal cells (100X).

FIGURE 27: SQUASH CYTOLOGY IN NON NEOPLASTIC LESIONS

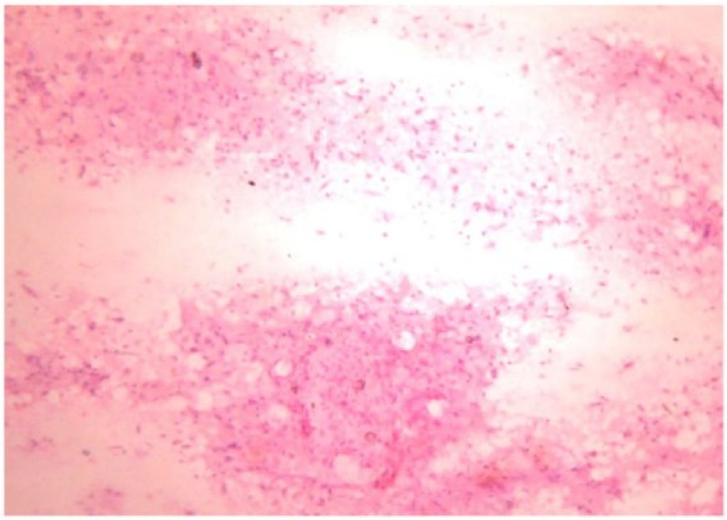

Figure 27: Smear with bland glial cells and scattered inflammatory cells (100X).

FIGURE 28: HISTOPATHOLOGY IN NON NEOPLASTIC LESION

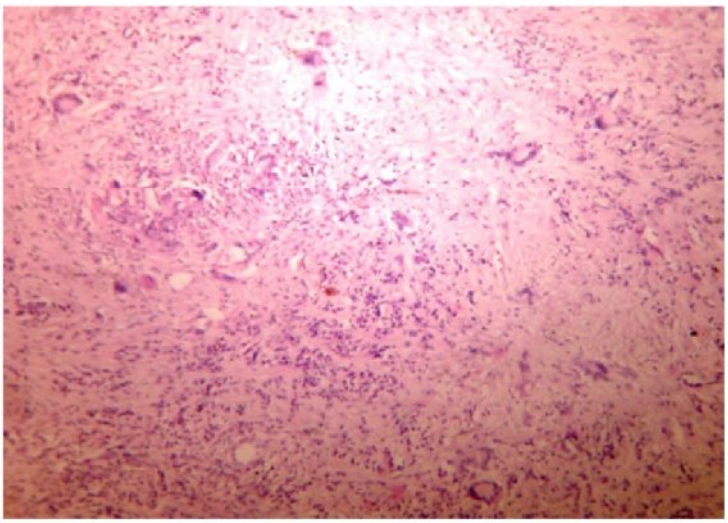

Figure 28: Inflammatory lesion showing chronic inflammatory cells and multinucleated giant cells (100X).

FIGURE 29: GOMORI'S METHANAMINE SILVER STAINING IN ASPERGILLUS

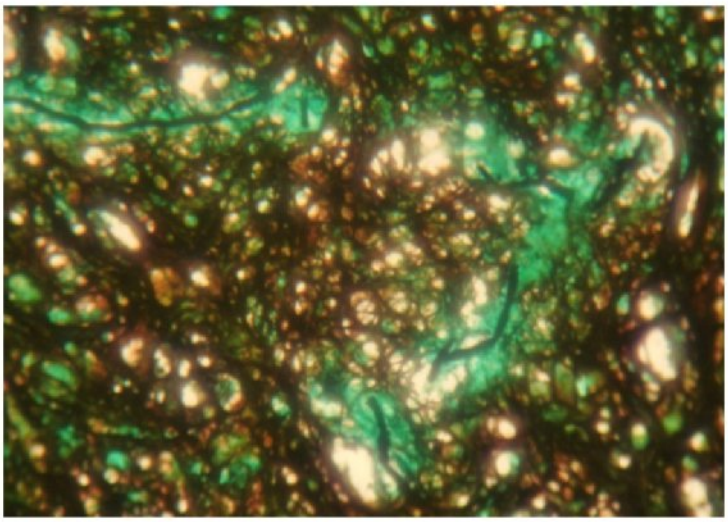

Figure 29: Acute, branching, septate hyphae of aspergillus are seen in gomori's methanamine silver staining 400X(GMS).

DISCUSSION

In this study, the general prevalence of CNS lesions reported at Department of Pathology, Coimbatore Medical College was found to be 1.1% (Chart.1).

TABLE 15. COMPARATIVE INCIDENCE OF CNS TUMORS IN INDIA

S.NO	CNS LESIONS	Incidence in the present study	Sumit et al study[44]	Kalpana et al study[45]
1	Astrocytoma	30%	44.55%	39.23%
2	Meningioma	26%	20%	19.13%
3	Neurofibroma/Schwannoma	12%	13.64%	11.96%
4	Oligodendroglioma	2%	2.7%	4.78%
5	Reactive Gliosis	4%	1.82%	2.87%
6	Infective lesions (Tuberculoma and Aspergillus)	8%	1.82%	3.82%
7	Medulloblastoma	2%	3.6%	3.35%
8	Ependymoma	2%	1.82%	1.91%
9	Hemangioblastoma	4%	2.7%	2.39%
10	CNS lymphoma	2%	1.82%	1.43%
11	Metastatic deposits	4%	3.6%	2.87%
12	Others (Keratocyst and Osteoma)	4%	1.82%	6.22%

In our study the incidence of astrocytoma are lower whereas the incidence of meningioma are high than that of Sumit[44] et al., and Kalpana[45] et al., study. Another population based study in west Bengal by Anirban[10] et al., showed the incidence of

astrocytoma to be 36.76% and meningioma to be 11.63%. The reason for such marked differences in incidence of astrocytoma and meningioma could be due to the lack of information about the disease pattern throughout India which creates problems in early detection at community level. Hence the exact incidence at community level cannot be determined.

However, the most common CNS tumor in India is astrocytoma followed by meningioma. This parallels with the worldwide incidence. In our study, among astrocytomas, grade 4 astroctoma was the most common tumor with an incidence of 53.33%. This correlates with other Indian studies as well as international studies[46].

COMPARATIVE ANALYSIS OF AGE DISTRIBUTION OF SPACE OCCUPYING LESIONS OF BRAIN AND SPINAL CORD

The age group in this study ranged from 6 years to 70 years with the mean age group of 39.56 years. The maximum incidence of CNS lesions occured in the 4th to 5th decades. This incidence parallels with worldwide statistics. The most common tumor in the 4th and 5th decade is astrocytoma. Among astrocytomas, glioblastoma multiformae occur mainly in the 5th decade with median age of 51 years. This parallels with studies of Monika[47] et al., and Sumit[44] et al., (Tables 2, 3 and 4).

There was one case of glioblastoma which occurred in the paediatric age group. It was a small cell variant of glioblastoma. Among tumors in the age group of 0 to 10 years, there were 2 cases of hemangioblastoma, 1 case of medulloblastoma, 1 case of pleomorphic xanthoastrocytoma and 1 case of small cell variant of glioblastoma.

According to Siegal Sadetzki[48] et al., meningiomas occur in the 4th and 5th decades of life. They actually show a bimodal age distribution with the first peak in 2nd decade and the second peak in the 4th decade. This parallels with the age incidence of meningioma in our study (Table 5).

COMPARISON OF GENDER DISTRIBUTION OF CNS LESIONS

In the present study, out of 50 cases, 26 were men and 24 were women with male to female ratio of 1.08:1. Astrocytoma was found to occur more commonly in men and meningioma, more commonly in women.

TABLE 16. COMPARISON OF GENDER DISTRIBUTION OF CNS LESIONS

S.NO	Tumor type	Anirban[10] et al (M:F)	Imtiaz[49] et al (M:F)	Present study (M:F)
1.	CNS tumors	1.71 : 1	2 : 1	1.08 : 1
2.	Astrocytoma	2.7 : 1	2.7 : 1	2 : 1
3.	Meningioma	0.74 : 1	1 : 2.8	0.44 : 1

Out of 42 cases of brain tumors, 36 cases occurred in the central nervous system and 6 cases occurred in the peripheral nervous system. Out of 36 cases in CNS, 22 cases occured in the middle cranial fossa which was the most common site of primary CNS tumors[50] (Table 6).

Squash cytology

Squash cytology is one of the methods employed for rapid diagnosis in neuropathology. Due to the intrinsic soft consistency of brain tissue, squash cytology is very useful in detecting CNS lesions. There are several advantages in squash cytology such as the speed and the ease of preparation, simplicity and preservation of cellular details. Details of cellular morphology are well preserved avoiding the freezing artefacts of cryostat sections. Most important advantage is that squash cytology requires only a small sample.

In the present study, 50 cases of space occupying lesions of brain and spinal cord were analysed using squash cytology and the results were compared with histopathology which is the golden standard.

Out of 50 cases, 42 were tumors and the remaining 8 were non neoplastic lesions. Among tumors, the diagnostic accuracy of squash cytology of the present study correlates well with other studies (Table 1).

TABLE 17. COMPARISON OF DIAGNOSTIC ACCURACY OF SQUASH CYTOLOGY IN CNS TUMORS

S.NO	CNS LESIONS	PRESENT STUDY	KALPANA STUDY[45]
11.	Astrocytoma	93.33% (n=15)	89.45% (n=82)
12.	Meningioma	84.62% (n=13)	87.5% (n=40)
13.	Neurofibroma/Schwannoma	83.33% (n=6)	100% (n=25)
14.	Oligodendroglioma	0% (n=1)	40% (n=10)
15.	Medulloblastoma	100% (n=1)	100% (n=7)
16.	Ependymoma	100% (n=1)	75% (n=4)
17.	Hemangioblastoma	50% (n=2)	100% (n=5)
18.	CNS lymphoma	100% (n=1)	100% (n=3)
19.	Metastatic deposits	50% (n=2)	100% (n=6)
20.	Others	25% (n=8)	50% (n=13)

ASTROCYTOMA

In the present study, 15 cases of astrocytoma were diagnosed. Out of these, squash cytology diagnosed 14 cases correctly. However correct grading was possible only in 11 cases. Hence the diagnostic accuracy without grading was 93.33% which decreased to 73.33% with grading. In fact it is inappropriate to grade astrocytoma based on squash cytology as astrocytoma vary significantly in grade from one area to another within a single tumor[45]. The squash smears were moderately cellular compared with normal brain tissue (Fig.1). The cells had elongated nuclei, irregular borders and coarse stippled chromatin (Fig. 2). Glioblastoma multiformae revealed vascular proliferation and mitosis (Fig. 5 & 6).

The diagnostic accuracy in grade 4 astrocytoma was only 62.5% compared with 80% for grade 2 and 100% for grade 3 astrocytomas (Chart 13). The reason is that the area of the tumor interpreted in squash cytology may not be representative to suggest grade 4 tumor such as necrosis or endovascular proliferation. One case could not be graded even in histopathology. Ki67/MIB1 labelling was performed for that case and the positivity was more than 10% suggesting the diagnosis of glioblastoma (Fig. 7).

In this study one case of small cell variant of glioblastoma was reported. This was diagnoses as medulloblastoma by radiology and squash cytology. The tumor cells were small and round. However histopathology revealed endovascular proliferation and necrosis suggesting the diagnosis of small cell glioblastoma. In children the differential diagnosis of small round cell tumors by squash cytology is difficult.

Pleomorphic xanthoastrocytoma

There were 2 cases of pleomorphic xanthoastrocytoma reported in the study. Both the cases were diagnosed correctly with squash cytology. Squash cytology revealed many multi nucleated giant cells and bizarre cells (Fig.23 & 24).

MENINGIOMA

Meningiomas were diagnosed easily with squash cytology. Out of the 13 cases, 11 were diagnosed correctly and 2 were missed. Out of the 2 cases, 1 case could not be interpreted due to unsatisfactory smear and 1 case was reported as Schwannoma as the fibrous areas mimicking antoni A areas of schwannoma. Spreading was easy for meningioma and on microscopy the cells appeared as intact whorls with many cases revealing psammoma bodies. The nuclei were round to oval and bland appearing (Fig.8 & 9). There were 3 cases of transitional meningioma which were reported as grade 1 meningioma by squash cytology (Fig. 10 & 11).

SCHWANNOMA AND NEUROFIBROMA

In this study 4 cases of schwannoma and 2 cases of neurofibroma were detected. Out of 6 cases, 5 cases were diagnosed correctly with diagnostic accuracy of 83.33% (Table 11). Schwannomas were firm and difficult to spread in a few cases. Schwannoma revealed fascicles of tumor cells with cellular antoni A areas (Fig. 12 & 13). S-100 staining in paraffin sections show strong and diffuse positivity in schwannoma (Fig. 14).

METASTATIC DEPOSITS

Two cases of metastatic carcinomatous deposits were detected in the study. One case was diagnosed correctly with squash cytology and the other was diagnosed as high grade glioma by squash cytology (Fig. 15 & 16). The diagnosis of metastasis in that case was confirmed in the paraffin sections using immunohistochemistry markers such as cytokeratin and glial fibrillary acidic protein. Carcinoma cells are glial fibrillary acidic protein negative[51] and cytokeratin positive.

OLIGODENDROGLIOMA

There was one case of Oligodendroglioma reported in the study which was misdiagnosed as grade 2 astrocytoma by squash cytology. According to Roessler et al., the diagnosis of Oligodendroglioma by squash cytology is difficult[50]. The peri nuclear halo in histopathology sections is absent in squash cytology which makes the diagnosis difficult (Fig. 17 & 18).

HEMANGIOBLASTOMA

Out of the 2 cases of Hemangioblastoma, one was diagnosed and another was missed. In Squash cytology hemangioblastoma were difficult to smear. The cytology showed thick tissue enclosing irregular vascular spaces (Fig. 19). In both the cases, reticulin staining in paraffin sections was useful in arriving at the final diagnosis. Reticulin staining in hemangioblastoma revealed the characteristic pericellular pattern (Fig. 21). CD 34 immunostaining revealed cytoplasmic positivity of endothelial cells (Fig. 22).

MEDULLOBLASTOMA AND EPENDYMOMA

One case of medulloblastoma and one case of ependymoma were diagnosed. Both were diagnosed with squash cytology with 100% diagnostic accuracy. Ependymoma was myxopapillary ependymoma with squash cytology showing papillary pattern and rosette like structures (Fig. 25 & 26). The differential diagnosis in elderly patients for small round cells tumors is CNS lymphoma.

PRIMARY CNS LYMPHOMA

There was one case of CNS lymphoma reported which was diagnosed correctly with squash cytology. Smear preparation revealed lack of cellular cohesion. The cells were large and pleomorphic in a dirty background.

NON NEOPLASTIC LESIONS

Non neoplastic lesions included 2 cases of tuberculoma, 2 cases of aspergillus, 2 cases of reactive gliosis, 1 case of keratocyst and 1 case of osteoma. Of these only 2 cases (1 case of reactive gliosis and 1 case of keratocyst) were detectable by squash cytology with a diagnostic accuracy of 25%. According to the study of Imtiaz[49] et al., the diagnostic accuracy in inflammatory lesion was only 50%. This is mainly due to the difficulty in making the smears as the tissue was rubbery. In our study, 1 case of osteoma and 1 case of aspergillus did not smear well. In osteoma the reactive gliosis area underneath the osteoma was sent for squash cytology. In aspergillus the tissue was firm and the diagnosis could not be established with histopathology. Only fungal staining gave the final diagnosis of Aspergillus (Fig 27, 28 & 29).

SENSITIVITY AND SPECIFICITY OF SQUASH CYTOLOGY

In this study the sensitivity of squash cytology was 97.62% and specificity was 75% which clearly implies that squash cytology is a valuble tool in CNS lesions (Table.14). The chi square value is 29.43 which is found to be significant at 1% level **(P<0.01)**. This shows that there is significant association between squash cytology and histopathology. Squash cytology is a reliable tool in detecting CNS lesions.

TABLE 18. DIAGNOSTIC ACCURACY OF SQUASH CYTOLOGY IN VARIOUS STUDIES – AN OVERVIEW

S.NO	NAME OF THE STUDY	DIAGNOSTIC ACCURACY IN PERCENTAGE
1.	Martinez[52] study	70%
2.	Kalpana[45] study	91.1%
3.	Imtiaz[49] study	84%
4.	Shaw[53] study	89.7%
5.	Sumit[44] study	88.5%
6.	Present study	74%

Hence squash cytology can be employed as method of rapid diagnosis of CNS lesions and can be used as an effective adjuvant in centres lacking intra operative frozen sections. However, it should be borne in mind that squash cytology is only a preliminary investigation and should not be used solely for diagnostic or therapeutic purposes. It has to be confirmed by histopathology.

SUMMARY

- The prevalence of CNS lesions reported in patients attending Coimbatore Medical College Hospital was 1.1 %.
- The most common tumor in CNS was astrocytoma (15 out of 42).
- The second most common tumor was meningioma (13 out of 42).
- Among Astrocytomas, glioblastoma multiformae constituted the most common group (8 out of 15).
- The most common age group of presentation for brain tumors was the 4^{th} and 5^{th} decade with the mean age group of 39.56 years.
- Glioblatoma multiformae occured mainly in the 5^{th} decade with the mean age group of 51 years.
- The male to female ratio was 1.08:1 for the CNS lesions in general.
- Astrocytomas showed male preponderance with male to female ratio of 2:1.
- Meningiomas showed female preponderance with male to female ratio of 0.44:1.
- Tumors of the central nervous system constituted 85.71% of tumors and the remaining cases occured in the peripheral nervous system. (36 out of 42).
- The most common site for CNS tumors was the middle cranial fossa (22 out of 36).
- Radiological investigations provided diagnostic accuracy of 60% (30 out of 50).
- Squash cytology had diagnostic accuracy of 74% (37 out of 50).
- The accuracy was higher for astrocytomas which constituted 93.33% (n=15).
- With grading of astrocytomas, the diagnostic accuracy decreased to 73.33% (11 out of 15).
- Diagnostic accuracy for meningioma was 84.62% (n=13).
- The diagnostic accuracy for neurofibroma and schwannoma was 83.33% (n=6).

- Medulloblastoma, ependymoma and CNS lymphoma had diagnostic accuracy of 100% (n=1 in each).
- Hemangioblastoma and metastatic deposits had diagnostic accuracy of 50% (n=2 in each).
- Oligodendroglioma could not be diagnosed with squash cytology.
- In non neoplastic lesions of CNS, the diagnostic accuracy was 25%.
- The sensitivity of squash cytology in detecting CNS tumors was 97.62%.
- The specificity of squash cytology in detecting CNS tumors was 75%.
- The positive predictive value of squash cytology was 95.35%.
- The negative predictive value of squash cytology was 85.71%.
- The percentage of false positive results was 25%.
- The percentage of false negative results was 2.38%.

CONCLUSION

Squash cytology is a sensitive and specific modality for diagnosing space occupying lesions of brain and spinal cord. The method is easy, rapid and inexpensive. Details of cellular morphology are well seen in squash cytology. Hence squash cytology can be used as a reliable diagnostic tool in developing countries like India, since the cost of cryostat is prohibitive and unlike cryostat, squash cytology does not require any electricity for slide preparation. Moreover, cryostat needs an experienced microtomist to cut the sections and the problems of freezing artefacts cause diagnostic pitfalls.

Despite the advantages, Squash cytology should be used as a preliminary investigation and should always be confirmed with Histopathology which is the golden standard. It should never be used solely for diagnostic or therapeutic purposes.

With increasing use of stereotactic biopsies, squash cytology provides means of assessing the tissue adequacy and also provides diagnosis rapidly. In the experienced hands of a pathologist with good exposure to neuropathology, squash cytology is an accurate and reliable procedure for rapid cytological diagnosis of CNS lesions.

ANNEXURE – I

PROFORMA

Coimbatore Medical College and Hospital, Coimbatore.

Name :

Age:

Sex:

IP No:

Income:

Occupation:

Study number:

History of presenting illness:

Duration of complaints:

Past history:

Family history:

Routine investigations:

CT diagnosis:

MRI diagnosis

Intra operative findings:

Squash diagnosis:

Histopathology diagnosis:

ANNEXURE II

Histological Classification of Brain Tumors – WHO (2007)

TUMOURS OF NEUROEPITHELIAL TISSUE

Astrocytic tumours

Pilocytic astrocytoma 9421/11

Pilomyxoid astrocytoma *9425/3**

Subependymal giant cell astrocytoma 9384/1

Pleomorphic xanthoastrocytoma 9424/3

Diffuse astrocytoma 9400/3

Fibrillary astrocytoma 9420/3

Gemistocytic astrocytoma 9411/3

Protoplasmic astrocytoma 9410/3

Anaplastic astrocytoma 9401/3

Glioblastoma 9440/3

Giant cell glioblastoma 9441/3

Gliosarcoma 9442/3

Gliomatosis cerebri 9381/3

Oligodendroglial tumours

Oligodendroglioma 9450/3

Anaplastic oligodendroglioma 9451/3

Oligoastrocytic tumours

Oligoastrocytoma 9382/3

Anaplastic oligoastrocytoma 9382/3

Ependymal tumours

Subependymoma 9383/1

Myxopapillary ependymoma 9394/1

Ependymoma 9391/3

Cellular 9391/3

Papillary 9393/3

Clear cell 9391/3

Tanycytic 9391/3

Anaplastic ependymoma 9392/3

Choroid plexus tumours

Choroid plexus papilloma 9390/0

Atypical choroid plexus papilloma *9390/1**

Choroid plexus carcinoma 9390/3

Other neuroepithelial tumours

Astroblastoma 9430/3

Chordoid glioma of the third ventricle 9444/1

Angiocentric glioma *9431/1**

Neuronal and mixed neuronal-glial tumours

Dysplastic gangliocytoma of cerebellum (Lhermitte-Duclos) 9493/0

Desmoplastic infantile astrocytoma/ ganglioglioma 9412/1

Dysembryoplastic neuroepithelial tumour 9413/0

Gangliocytoma 9492/0

Ganglioglioma 9505/1

Anaplastic ganglioglioma 9505/3

Central neurocytoma 9506/1

Extraventricular neurocytoma *9506/1**

Cerebellar liponeurocytoma *9506/1**

Papillary glioneuronal tumour *9509/1**

Rosette-forming glioneuronal tumour of the fourth ventricle *9509/1**

Paraganglioma 8680/1

Tumours of the pineal region

Pineocytoma 9361/1

Pineal parenchymal tumour of intermediate differentiation 9362/3

Pineoblastoma 9362/3

Papillary tumour of the pineal region *9395/3**

Embryonal tumours

Medulloblastoma 9470/3

Desmoplastic/nodular medulloblastoma 9471/3

Medulloblastoma with extensive nodularity *9471/3**

Anaplastic medulloblastoma *9474/3**

Large cell medulloblastoma 9474/3

CNS primitive neuroectodermal tumour 9473/3

CNS Neuroblastoma 9500/3

CNS Ganglioneuroblastoma 9490/3

Medulloepithelioma 9501/3

Ependymoblastoma 9392/3

Atypical teratoid / rhabdoid tumour 9508/3

TUMOURS OF CRANIAL AND PARASPINAL NERVES

Schwannoma (neurilemoma, neurinoma) 9560/0

Cellular 9560/0

Plexiform 9560/0

Melanotic 9560/0

Neurofibroma 9540/0

Plexiform 9550/0

ANNEXURE III

SPECIAL STAINS

RETICULIN STAIN

PROCEDURE

1. Deparaffinize sections and bring to water.
2. Treat with 1%potassium permanganate solution -1min(the adjacent hydroxyl groups of the hexose sugars of glycoproteins are oxidized to aldehydes by potassium permanganate).
3. Rinsed in tap water.
4. Bleached in 2%potassium metabisulfate solution-1 min,then rinsed in tap water.
5. Sensitized with ferric ammonium sulphate – 1 min and washed in distilled water.
6. Impregnated in silver solution (containing silver nitrate , KOH, and ammonia) for 1 min.
7. Washed in distilled water several times.
8. Reduced in 20% formalin solution for 3 minutes(local silver reaction is amplified to produce visible silver deposits). Then rinsed in tap water.
9. Toned in 0.2 % gold chloride for 10 minutes. Rinsed again in tap water.
10. Treated with 2% potassium metabisulfite solution for 1 min. Rinsed again in tap water.
11. Treated with 2% sodium thiosulfate solution for 1 min . Rinsed again in tap water.
12. The section is counterstained with eosin so the defined reticulin fibres appear black.

    Results:

    Reticulin fibres – Black.

    Background – colourless.

FUNGAL STAINING – GOMORI'S METHANAMINE SILVER STAINING

PROCEDURE

1. Sections to water.
2. Put sections in 5% chromic acid for 1 hour.
3. Wash sections in tap water for 10 seconds.

4. Rinse in potassium metabisulfite for 1 min (to remove residual chromic acid).
5. Wash in tap water for 10 minutes.
6. Rinse in distilled water.
7. Place in freshly prepared methanamine silver solution for 1 to 2 minutes at 58-60^0 celcius in microwave oven till the sections turn yellowish brown.
8. Rinse in distilled water.
9. Tone in gold chloride for 5 minutes.
10. Rinse in distilled water.
11. Remove the unreduced silver with sodium thiosulphate for 5 minutes.
12. Wash thoroughly in tap water.
13. Counterstain with light green for 3 minutes with microscopic control.
14. Dehydrate in alcohol.
15. Clear in xylene.
16. Mount in DPX.

Results:

Fungus – Black.

Background – Green.

IMMUNOHISTOCHEMISTRY

PROCEDURE

1. Bring sections in water.
2. Rinse briefly in distilled water.
3. Keep in microwave in medium for 10 minutes and high for 10 minutes.
4. Cool to room temperature.
5. Rinse in distilled water for 5 minutes.
6. Wash in Tris buffer saline for 5 minutes – 2 changes.
7. Cool to room temperature for 20 minutes.
8. Rinse in distilled water for 5 minutes.
9. Wash in Tris buffer saline for 5 minutes – 2 changes.
10. Keep in peroxide block for 10 minutes.
11. Wash in Tris buffer saline for 5 minutes – 2 changes.
12. Keep in power block in 10 minutes.
13. Drain sections and cover with primary antibody for 1 hour.

14. Wash in Tris buffer saline for 5 minutes – 2 changes.
15. Keep sections in super enhancer for 30 minutes.
16. Wash in Tris buffer saline for 5 minutes – 2 changes.
17. SS labeling with poly HRP for 30 minutes.
18. Wash in Tris buffer saline for 5 minutes – 2 changes.
19. Keep in diamino benzidine (DAB) and substrate buffer for 5 minutes.
20. Wash in Tris buffer saline for 5 minutes – 2 changes.
21. Wash in tap water for 5 minutes.
22. Keep sections in haematoxylin for 30 seconds.
23. Wash in tap water for 5 minutes.
24. Air dry, dehydrate, clear in xylol and mount in DPX.

Results:

The development of brown color is taken as positive.

ANNEXURE – IV
MASTER CHART

S.NO	PATIENT NAME	IP No	AGE	SEX	CT DIAGNOSIS	SITE	SQUASH CYTOLOGY DIAGNOSIS	GRADE	HISTOPATHOLOGY DIAGNOSIS	GRADE
01	Tamilselvi	61758	28	F	Neurofibroma	C5-C6 Vertebra	Neurofibroma		Neurofibroma	
02	Pachainayagi	64202	39	F	Oligodendroglioma	Frontal lobe	Glioma	2	Oligodendroglioma	2
03	Arumugam	66657	55	M	High grade Glioma	Lt Temporal lobe	Glioma	3	Glioblastoma Multiforme	4
04	Rahim	63668	70	M	High grade glioma	Rt Frontal lobe	Metastatic adenocarcinomatous deposits		Poorly differentiated carcinomatous deposits	
05	Ranjith kumar	67782	6	M	Cerebral abscess	Rt Temporal lobe	Inflammatory lesion with Reactive gliosis/?low grade glioma		Reactive gliosis	
06	Mani	63947	42	M	Primary CNS Lymphoma	B/L Carpus Callosum	High grade Non hodgkins lymphoma		Non Hodgkins lymphoma-Diffuse large B cell type	

07	Jayalakshmi	69660	56	F	Acoustic Neuroma	CP angle	Schwannoma		Schwannoma	
08	Ramathal	69024	52	F	Atypical Falx Meningioma	Falx cerebri	Meningioma	1	Transitional cell Meningioma	1
09	shobana	69654	30	F	Pituitary Adenoma	Pitutary Stalk and Optic Chaisma	Unsatisfacory smear		Transitional cell Meningioma	1
10	Logapriya	71755	10	F	PNET	Cerebellar vermis	Medulloblastoma		Hemangioblastoma	
11	Ranjini	72898	23	F	Low grade glioma	Rt Frontal lobe	Meningioma	1	Transitional cell Meningioma	1
12	Subramani	74669	60	M	High grade Glioma	Rt Parietal Lobe	Gliobastoma Multiforme	4	Gliobastoma Multiforme	4
13	Krishnan	75542	54	M	High grade Glioma	Lt Parieto occcipital lobe	Gliobastoma Multiforme	4	Gliobastoma Multiforme	4
14	Selvi	71919	45	F	Neurofibroma	Cervical spine	Neurofibroma		Neurofibroma	
15	Karunapillai	75597	60	M	Low grade meningioma	D9 Vertebra	Meningioma	1	Transitional meningioma	1

16	Ganndasamy	76721	34	M	Pilocytic Astrocytoma	Rt Parieto Temporal Region	Low Grade Glioma	1	Diffuse Astrocytoma	2
17	Mohan	4950	45	M	Neurofibroma	Sacro Coccygeal Region	Ependymoma		Myxopapillary Ependymoma	
18	Mariyammal	3346	42	F	Low grade Glioma/ Tuberculoma	Rt Parietal cortical Region	Gliobastoma Multiforme	4	Gliobastoma Multiforme	4
19	Sarumathi	6366	21	F	Benign Lesion - Teratoma	Lt Parietal SOL	Keratocyst		Keratocyst Epidermoid Type	
20	Lakshmi	9854	50	F	High grade Glioma	Rt Fronto Parietal SOL	Gliobastoma Multiforme	4	Gliobastoma Multiforme	4
21	Chandrasekar	11790	36	M	Low grade glioma	Rt Frontal Region	High Grade Glioma Grade	3	Glioma	3
22	Rangasamy	13054	62	M	High grade Glioma	Posterior Parietal SOL	High Grade Glioma	3/4	Metastatic AdenoCarcinomatous Deposits	
23	Vadivel	17147	33	M	Aspergillosis	Lt Frontal SOL	Bland glial cells, inflammatory cells, No fungal hyphae		?Fungal infection/?TB, GMS-Aspergillus	

24	Ashok Kumar	17930	20	M	Medulloblastoma	Cerebellar vermis	Small Round Cell Tumor		Tuberculous Lesion	
25	Gowri	21897	49	F	Meningioma	Rt Parietal Convexity	Meningioma	1	Psammomatous Meningioma	1
26	Prakash	21330	9	M	Medulloblastoma	Cerebellum	Medulloblastoma		Medulloblastoma	
27	Suresh Kumar	24630	46	M	Low Grade Glioma	Rt Parieto Occipital SOL	Diffuse Astrocytoma	2	Diffuse Astrocytoma	2
28	Ayyavu	24008	57	M	High Grade Glioma/Metastasis	Rt temporal Region	Diffuse Astrocytoma	2	Diffuse astrocytoma	2
29	Sundari	25245	45	F	Meningioma	Rt temporo parietal region	Meningioma	1	Meningotheliall meningioma	1
30	Kalamani	25908	27	F	Neurofibroma/Schwannoma	T10-T11	Schwannoma		Fibrous meningioma	1
31	Baggyalakshmi	28290	62	F	Low grade Glioma	Fronto parietal SOL	Pleomorphic Xantho Astrocytoma	2	Pleomorphic Xantho Astrocytoma	2
32	Sujitha	30777	9	F	Low Grade Glioma	III ventricular SOL	Hemangioblastoma		Hemangioblastoma	

33	Kadhar	29758	45	M	Schwannoma	D8-D9 compression	Neurofibroma		Schwannoma Antoni A type	
34	Karunanidhi	32870	52	M	Glioblastoma Multiformae	Rt temporp parietal region	Low grade Glioma	1	Glioblastoma Multiformae	4
35	Chitra	31269	45	F	Meningioma	Supra sellar SOL	Meningothelial meningioma	1	Meningothelial meningioma	1
36	Kanniyammal	33495	58	F	meningioma	Lt temporo parietal SOL	Meningothelial maningioma	1	Meningothelial meningioma	1
37	Arumugam	34582	45	M	Glioblastoma multiformae	Rt temporo parietal SOL	Glioblastoma multiformae	4	Glioblastoma multiformae	4
38	Rajamani	33508	48	F	Schwannoma	Rt cerebello pontine angle tumor	Schwannoma		Schwannoma	
39	Valli	32942	57	F	Cystic Schwannoma	Lt cerebello pontine angle tumor	Schwannoma		Schwannoma	
40	Valliammal	37688	35	F	Low grade glioma	Lt fronto parietal SOL	Astrocytoma	3	Astrocytoma	3

41	Palanisamy	39938	43	M	Low grade glioma	Lt thalamic SOL	Atypical meningioma	2	Atypical meningioma	2
42	Subban	40496	28	M	meningioma	Lt frontal SOL	Psammomatous meningioma	1	Psammomatous meningioma	1
43	Karuppammal	44043	45	F	Meningioma	Lt parietal SOL	Meningioma	1	Meningioma	1
44	Manikandan	46037	37	M	Meningioma	Lt frontal SOL	Unsatisfactory smear		Fungal Lesion, GMS-Aspergillus	
45	Ramachandran	47380	30	M	Calcified Meningioma	Rt Parietal SOL	Reactive gliosis		Osteoma	
46	Anandhi	48841	9	F	Low grade glioma	Rt Parietal SOL	Pleomorphic Xantho Astrocytoma	2	Pleomorphic Xantho Astrocytoma	2
47	Selvam	46048	31	M	Tuberculous abscess	III ventricular SOL	Inflammatory lesion		Koch lesion	
48	Adithyan	50519	6	M	PNET	Rt parietal parasagital SOL	Medulloblastoma		Small cell variant of glioblastoma multiformae	4
49	Kalliyammal	46724	47	F	High grade glioma	Rt Thalamic SOL	Diffuse astrocytoma	2	Reactive gliosis	
50	Ramasamy	38994	40	M	Meningioma	Parieto occipital SOL	Meningioma	1	Meningothelial meningioma	1

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